SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF 2-AZETIDINONE AND 4-THIAZOLIDINONE DERIVATIVES

Dissertation Submitted to

The Tamil Nadu Dr. M.G.R. Medical University,

Chennai – 600 032.

In partial fulfillment for the award of Degree of

MASTER OF PHARMACY

(PHARMACEUTICAL CHEMISTRY)

Submitted by

S. SETHUVANI

Reg.No:26106036

Under the Guidance of

Mr. M. SENTHIL @ PALANIAPPAN, M. Pharm.,

Professor

Department of Pharmaceutical Chemistry

&

Mr. M. SUGUMARAN, M. Pharm., (Ph.D.)

Associate Professor

Department of Pharmaceutical Chemistry

ADHIPARASAKTHI COLLEGE OF PHARMACY

(Accredited By "NAAC" with CGPA of 2.74 on a Four Point Scale at "B" Grade)

MELMARUVATHUR-603 319

MAY – 2012

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CERTIFICATE

This is to certify that the dissertation entitled **"SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF 2-AZETIDINONE AND 4-THIAZOLIDINONE DERIVATIVES"** submitted to The Tamil Nadu Dr. M.G.R. Medical University, Chennai, in partial fulfillment for the award of the Degree of the Master of Pharmacy (Pharmaceutical Chemistry) was carried out by **S. SETHUVANI (Register No.26106036)** in the Department of Pharmaceutical Chemistry under our direct guidance and supervision during the academic year 2011-2012.

Place: Melmaruvathur
Date:

Mr. M. SENTHIL@PALANIAPPAN, M. Pharm.,
Professor,
Department of Pharmaceutical Chemistry,
Adhiparasakthi College of Pharmacy,
Melmaruvathur – 603 319.

Place: Melmaruvathur
Date:

Mr. M. SUGUMARAN, M. Pharm., (Ph.D.)
Associate Professor,
Department of Pharmaceutical Chemistry,
Adhiparasakthi College of Pharmacy,
Melmaruvathur – 603 319.

CERTIFICATE

This is certify that the dissertation entitled **"SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF 2-AZETIDINONE AND 4-THIAZOLIDINONE DERIVATIVES"** is the bonafide research work carried out by **S. SETHUVANI (Register No.26106036)** in the Department of Pharmaceutical Chemistry, Adhiparasakthi College of Pharmacy, Melmaruvathur, which is affiliated to The Tamilnadu Dr. M.G.R Medical University under the guidance of **Mr. M. SENTHIL @ PALANIAPPAN, M.Pharm.,** Professor and co-guidance of **Mr. M. SUGUMARAN, M.Pharm., (Ph.D.),** Associate Professor, Department of Pharmaceutical Chemistry, Adhiparasakthi College of Pharmacy, during the academic year 2011-2012.

Place: Melmaruvathur
Date:

Prof. (Dr.) T.VETRICHELVAN, M. Pharm., Ph.D.,
Principal,
Adhiparasakthi College of Pharmacy,
Melmaruvathur – 603 319.
Tamilnadu.

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I humbly present this work to the eternal **ALMIGHTY.** Indeed our project is a small work done with the help of primitive persons at heart. So it is our bounded duty to promulgate them individually.

With great respect and honor, I extend my thanks to **THIRUMATHI LAKSHMI BANGARU ADIGALAR,** Vice President, ACMEC Trust, Melmaruvathur for given me an opportunity and encouragement all the way in completing the study. Her excellence in providing skillful and compassionate spirit of unstinted support to our department for carrying out dissertation entitled "**SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF 2-AZETIDINONE AND 4-THIAZOLIDINONE DERIVATIVES.**"

The research work embodied in dissertation has been carried out under supervision of my esteemed and most respected guides **Mr. M. SENTHIL @ PALANIAPPAN**, **M.Pharm.,** Professor and **Mr. M. SUGUMARAN, M.Pharm., (Ph.D.),** Associate Professor, Department of Pharmaceutical Chemistry Adhiparasakthi College of Pharmacy for the active guidance, valuable suggestions and a source of inspiration where the real treasure of my work.

I owe my sincere thanks with bounteous pleasure **Prof. (Dr.) T.VETRICHELVAN, M.Pharm., Ph.D.** Principal, Adhiparasakthi College of Pharmacy, without his

encouragement and supervision, it would have been absolutely impossible to bring out the work in this manner.

I express my deep sense of gratitude to respected **Mrs. D. NAGAVALLI, M. Pharm., Ph.D.,** Professor, **Mr. A. THIRUGNANASAMBANTHAN, M. Pharm., (Ph.D.),** Assistant Professor**,** Department of Pharmaceutical Chemistry, **Mr. K. ANANDAKUMAR, M. Pharm., (Ph.D),** Associate Professor, Department of Pharmaceutical Analysis and other faculty members of Adhiparasakthi college of Pharmacy, Melmaruvathur, for their valuable help and guidance during the course of my research work.

I am extremely gratified to **Mrs. S. SHOBA**, **M. Pharm.,** Assistant Professor and **Ms. M. VIJAYAKUMARI, M. Pharm.,** Lecturer, Department of Pharmacology, Adhiparasakthi College of Pharmacy, Melmaruvathur, for their invariable support, assistance and guidance to carrying out the pharmacological studies.

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I am indeed very much thankful to the librarian **Mr. M. SURESH, M.L.I.S.,** Adhiparasakthi College of Pharmacy, for providing all reference books for the completion of this project.

I wish to regard my heartily thanks to **Dr. R. MURUGESAN,** Scientific Officer-I, Sophisticated Analytical Instrument Facility, Indian Institute of Technology Madras, Chennai-600036, for taking IR, NMR and MASS Spectroscopy studies.

I extend my heartily thanks to **Mr. K. MARUTHAPPAPANDIAN**, Ideal Analytical and Research Institution, Puducherry – 605 110, for taking IR Spectroscopy studies and also thankful to **Mr. AVATAR SINGH**, Executive Officer, SAIF, Punjab University, Chandigarh, for taking NMR spectroscopy.

I am thankful to all my class friends, juniors and my friends for their support and suggestion during my work.

Finally yet importantly, I gratefully forward my affectionate thanks to my family members, especially **my parents** and **sister** for their frequent prayers, which has sustained me a lot in the successful completion of my project work**.**

S. SETHUVANI

Dedicated To

My Beloved Parents

&

All My Friends... 

CONTENTS

LIST OF ABBREVATIONS

^0C	:	Degree Centigrade
g	:	Gram
mg	:	Milligram
ml	:	Milliliter
m.p	:	Melting point
pH	:	Hydrogen ion concentration
h	:	Hours
min	:	Minutes
mol	:	Mole
µg	:	Microgram
%	:	Percentage
cm	:	Centimeter
DMF	:	Dimethyl formamide
DMSO	:	Dimethyl sulphoxide
^1H-NMR	:	Proton Nuclear Magnetic Resonance
IR	:	Infra Red
QSAR	:	Quantitative Structural Activity Relationship
UV	:	Ultra Violet
TLC	:	Thin Layer Chromatography
Ar	:	Aromatic
Alip.	:	Aliphatic
DMSO-d_6	:	Deuterated Dimethyl sulphoxide
Me	:	Methyl

Et	:	Ethyl
HIV	:	Human Immunodeficiency Virus
TEA	:	Triethyl amine
MeOH	:	Methanol
EtOH	:	Ethanol
Ph	:	Phenyl
o, m. p	:	Ortho, Meta, Para
δ	:	Delta
ppm	:	Parts per million
m/z	:	Mass / charge
R_f	:	Retention factor
Str.	:	Stretching
S. aureus	:	*Staphylococcus aureus*
E.coli	:	*Escherichia coli*
C. albicans	:	*Candida albicans*
ED_{50}	:	Effective Dose
KBr	:	Potassium bromide
ATCC	:	American Type Culture Collection
kg	:	Kilogram
LD_{50}	:	Lethal Dose
ANOVA	:	Analysis of Variance
CFU	:	Colony Forming Unit
gram +ve	:	Gram positive
gram –ve	:	Gram negative
µl	:	Microlitre

CPCSEA	:	Committee for the Purpose of Control and Supervision of Experiments on Animals
M^+	:	Molecular ion
nm	:	Nanometer
OECD	:	Organization for Economic Cooperation and Development
PEG	:	Poly Ethylene Glycol
SEM	:	Standard Error of Mean
IAEC	:	Institutional Animal Ethical Committee
Sec	:	Seconds
s	:	Singlet
d	:	Doublet
m	:	Multiplet
w/v	:	weight/volume

INTRODUCTION

1. INTRODUCTION

Medicinal Chemistry has its roots in several branches of chemistry and biology. It is concerned with the study, identification and synthesis of the metabolic products of drugs and related compounds. It also attempts to establish relationship between structure and function and to link biodynamic behavior with chemical reactivity and physical properties. However, essentially it concerns with the understanding of mechanisms of action of drugs. Besides this, medicinal chemistry also involves the isolation, characterization and synthesis of compounds that can be used in medicine for the prevention, treatment and cure of diseases. Thus, it provides chemical basis for the interdisciplinary field of therapeutics.

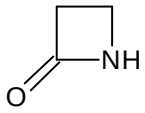
The primary objective of medicinal chemistry is the design and discovery of new compounds that are suitable for use as drugs. The discovery of a new drug requires not only its design and synthesis but also the development of testing methods and procedures, which are needed to establish how a substance operates in the body and its suitability for use as a drug. Drug discovery may also require fundamental research into the biological and chemical nature of the diseased state. This and other aspects of drug design and discovery require input from specialists in other fields, such as biology, biochemistry, pharmacology, mathematics, computing and medicine amongst others, and the medicinal chemist to have outline knowledge of these fields.

2-Azetidinone and 4-thiazolidinone have played an important role in medicinal chemistry. Moreover they have been studied extensively because of their ready accessibility, diverse chemical reactivity and broad spectrum of biological activity.

1.1. 2-AZETIDINONE

2-Azetidinone, commonly known as β-lactams, is a four member cyclic amide which was first synthesized by Staudinger in 1907. They are the carbonyl derivatives of azetidines containing carbonyl group at the 2nd position.

Structure:



Molecular formula : C_3H_5NO

Molecular weight : 71.08

Synonym : 2-Azacyclobutanone, Propiolactams

Physical properties:

Description : Colourless solid

Melting point : 73-74º C

Boiling point : 106º C

Density : 1.119 g/cm^3

Molar refractivity : 17.314 cm^3

Solubility : Soluble in ethanol, acetone; slightly soluble in benzene

Stability : Stable under normal temperature and condition

The 2-azetidinone (β- lactams) ring is a common structural feature of a number of broad spectrum β-lactam antibiotics including penicillins, cephalosporins, carbapenems, nocardicin and monobactams which have been widely used as chemotherapeutic agents to treat bacterial infection and microbial diseases. Apart from antibiotic activity β- lactam also possess cholesterol inhibition, antithrombotic, antiviral and antitumor activities. (Mrunmayee Toraskar. *et al.,* **2010**)

It has been reported that introduction of different substituents to four membered β-lactam nucleus tend to exert profound influence in conferring promising

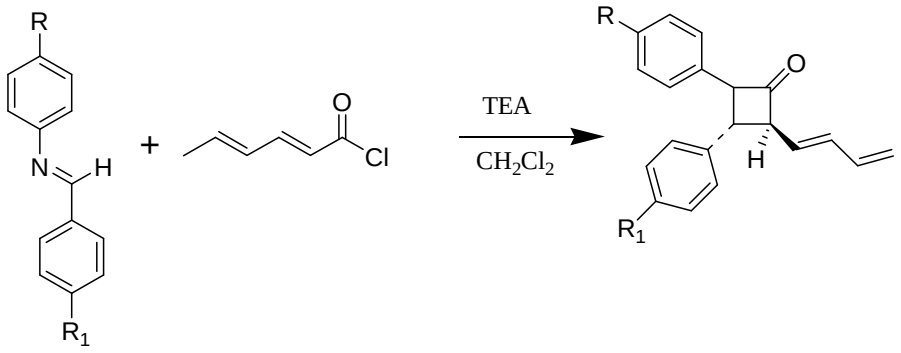
biological activities. A large number of 3-chloro monocyclic β-lactam possesses powerful antibacterial, antimicrobial, anti-inflammatory, anticonvulsant & antitubercular activities. They also function as enzyme inhibitors & are effective on the central nervous system (Ameya A.Chavan. *et al.*, **2007**). Subsequently 2-azetidinones were highlighted as a potent mechanism based inhibitor of several enzymes like human tryptase, chymase, thrombin, leukocyte elastase, human cytomegalovirus protease and serine protease enzyme. These derivatives are also known to possess antitubercular, anti-inflammatory, antitumor, anti-HIV, antiparkinsonism, antidiabetic and vasopressin antagonist activity. (Mehta PD. *et al.*, **2010**)

Synthetic methods of azetidinones

Many methods had been reported for the synthesis of 2-azetidinones in the literature. Some of those methods were listed in this study.

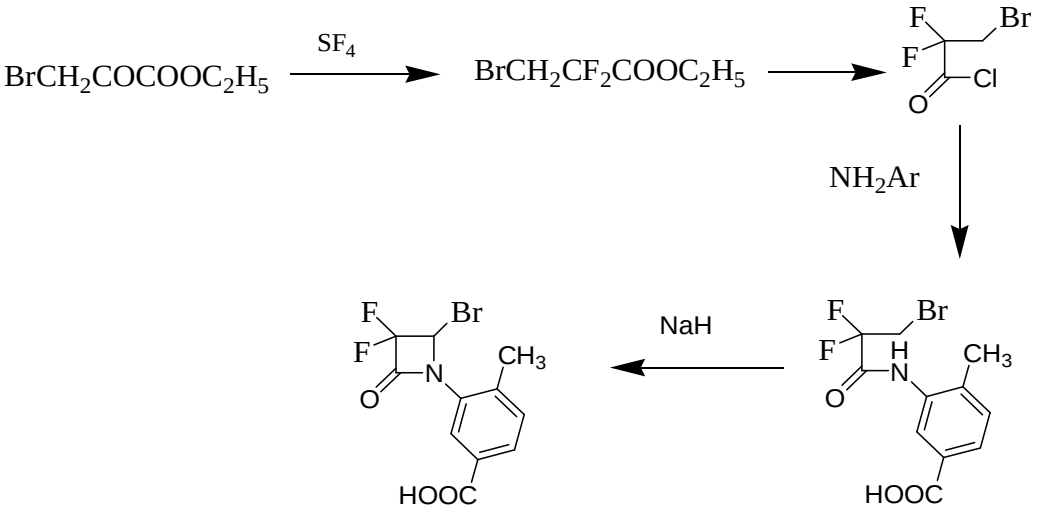
a) Ketene-imines Cycloaddition

The ketene-imines cycloaddition was reported to be a smooth well-documented route to the synthesis of substituted β - lactam derivatives. In an effort to investigate suitably substituted monocyclic β-lactam as a minimum requirement for biological activity, many scientists reported the trans-stereoselective synthesis of butadienyl azetidinones and their Diels-Alder cycloaddition. This included the preparation of a series of Schiff's bases and their reaction with dienylketene to produce a trans-azetidinone .This involved the in situ formation of the ketene and its subsequent addition to the imines. (Staudinger H. *et al*., **1912**)



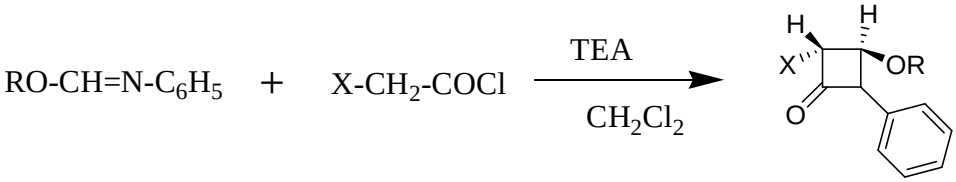
b) Wasserman Cyclization

The stability of 2-azetidinones to enzymatic ring opening by β-lactamases was studied. They suggested that a halogen ß- to the carbonyl would increase the IR absorption of the C=O, one of the criteria of the reactivity of the β-lactam in this aspect. Fluorine substitution, which will not introduce a large steric hindrance, is particularly interesting for a possible biological effect and possible stability towards β-lactamase. β-Bromopropionamide derivative was prepared, which can be cyclized by wasserman procedure using sodium hydride to give the N-(3-carboxy-6-methylphenyl) 3- difluoro-2-azetidinone. (Roger Joyeau. *et al.*, **1988**)



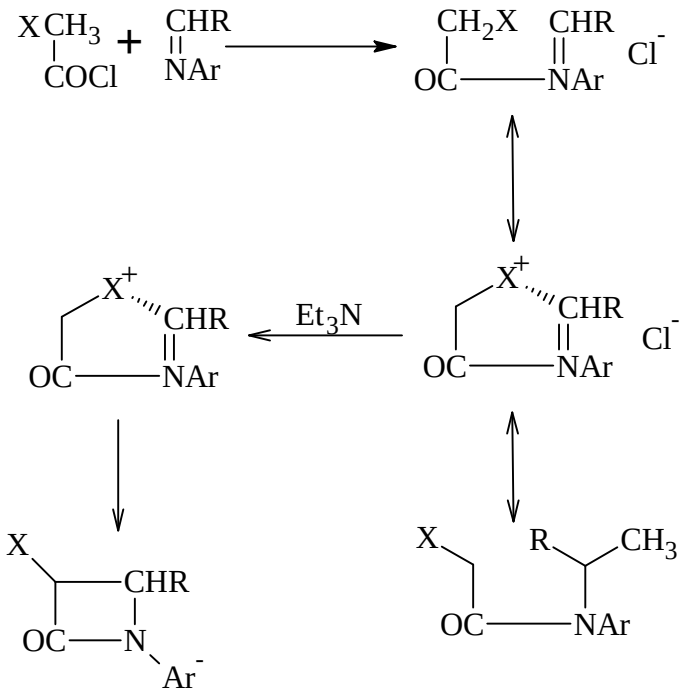
c) Synthesis of ß-Lactams from Imidates

The synthesis of alkoxy β–lactams via acid chloride imine route was reported. Imidates such as substituted N-phenyl formimidate, were reacted with acid chlorides to produce β-lactam. The major feature of this synthesis is its high stereoselectivity, only trans-4-alkoxy- β-lactams were formed. (Cardellini. *et al.*, **1984**)



d) Acid Chloride Addition Reaction

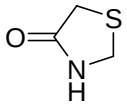
A method to prepare β-lactams which involved the reaction between benzylideneaniline and few selected acid was reported. (Bose. *et al.*, **1995**)



1.2. 4-THIAZOLIDINONE

4-Thiazolidinones are derivatives of thiazolidine with a carbonyl group at the 4-position, has been considered as a moiety of choice as it possesses a broad spectrum of pharmacological activities against several targets.

Structure:



Molecular formula : C_3H_5NOS

Molecular weight : 103.143

Synonyms : 1, 3-thiazolidin-4-one

Physical properties

Description : Colourless solid

Melting point : 194° C

Boiling point : 294.52° C

Density : 1.278 g/cm^3

Molar refractivity : 25.476 cm^3

Solubility : Soluble in ethanol, acetone; insoluble in water

Stability : Stable under normal temperature and condition

4-Thiazolidinone ring also occurs in nature; thus actithiazic acid [(–) 2-(5-carboxypentyl) thiazolidin-4-one)] isolated from *Streptomyces* strains exhibits highly specific *in vitro* activity against *Mycobacterium tuberculosis.* (Saeed. *et al.,* **2007**)

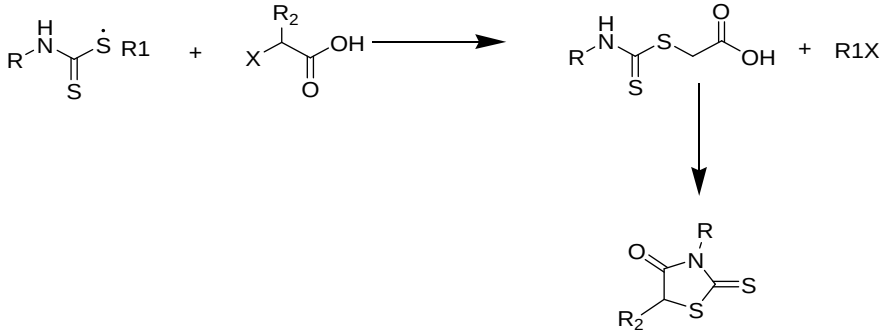
Thiazolidinone derivatives are the subject of renowned interest because they have been found to be useful intermediates for the synthesis of various heterocyclic compounds. 4-Thiazolidinone derivatives has been considerable interest in the chemistry of thiazolidin-4-one ring systems, which is a core structure in various

synthetic pharmaceuticals displaying a broad spectrum of biological activities which includes anti-inflammatory, anticonvulsant, anti-fungal, anti-thyroid, antitubercular and antidiabetic. Antimicrobial activity is the most potent activity of thiazolidine-4-one. Antibacterial activity is strongly dependent on the nature of substituent at C-2 and N-3 position. It was also found to possess various other activities such as antidiarrheal, antiplatelet activating factor, antihistaminic, antimicrobial, antidiabetic, cycloxygenase inhibitory, Ca2+ channel blocker, PAF antagonist, cardioprotective, anti ischemic, anti cancer, anti HIV, non-peptide thrombin receptor antagonist and Follicle stimulating hormone (FSH) receptor agonist activity and CFTR inhibitor activity. (Mulay abhinit. *et al.,* **2009**)

Synthetic methods of 4-thiazolidinone

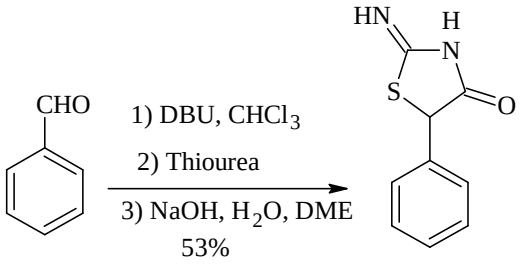
Several methods for synthesis are available in literature which involves conventional one pot, two pot synthesis and microwave as well as combinatorial syntheses methods.

a) The dithiocarbamates formed by the reaction of primary amine with carbon disulfide in the presence of base react with haloalkanoic acid in the presence of $NaHCO_3$ to give substituted 2-thiono-4- thiazolidinones as presented in the scheme. (Cunico W. *et al.,* **2007**)

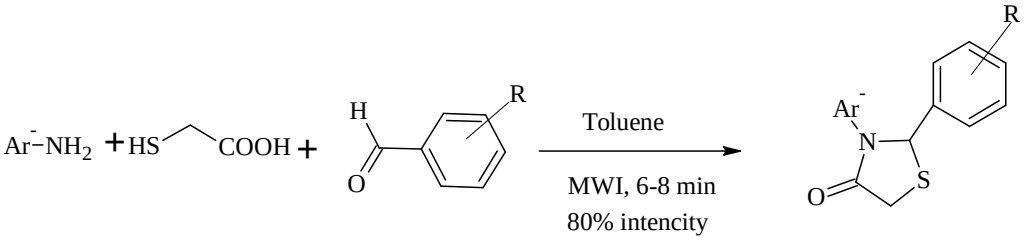


b) One pot three component synthesis containing aldehyde, thiourea and chloroform to give 2-amino-4-thiazolidinone derivatives was also reported. Various imino

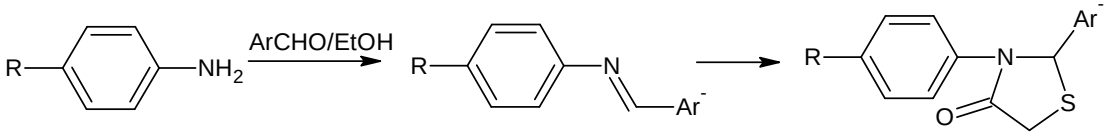
thiazolidinones were developed by using different reagents with different reaction conditions. (Jieping Z. *et al.,* **2004**)



c) The synthesis of the 2,3-diaryl-1,3-thiazolidin-4-ones was reported. It was done by reacting substituted benzaldehyde with equimolar amount of an appropriate substituted aromatic amine in the presence of an excess of mercaptoacetic acid in toluene utilizing microwave irradiation. (Sriram D. *et al.,* **2005**)

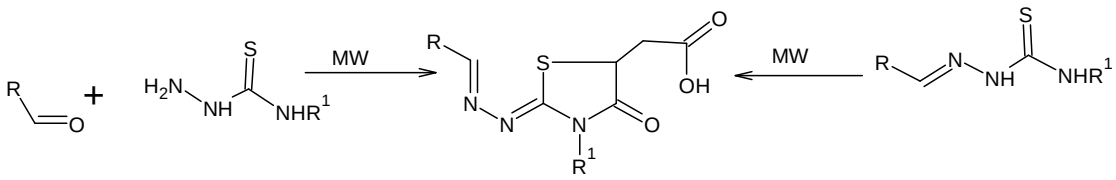


d) A series thiazolidin-4-one was synthesized by the reaction with schiff bases. The Schiff bases were synthesized by condensation of aromatic amine with different substitute aromatic aldehydes. The obtained Schiff bases were subjected to condensation with mercaptoacetic acid to give the corresponding 4-thiazolidinones. (Jubie. *et al.,* **2009**)

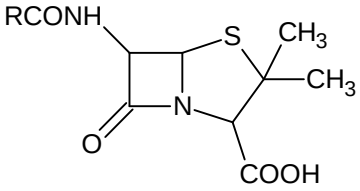


e) An efficient tandem procedure for the synthesis of 2-hydrazolyl-4-thiazolidinones under microwave conditions was reported. Different solvents and various reaction equivalents were explored until good isolated yields of thiazolidinones were obtained.

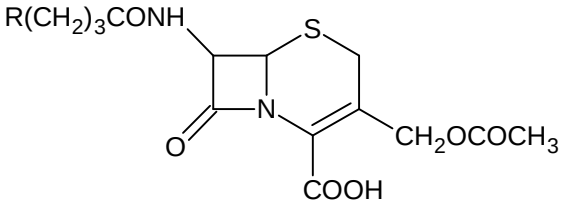
Microwave heating for the synthesis of thiazolidinone resulted in a significantly better yield compared to thermal conditions (75% vs 40%). Microwave irradiation also allowed for a faster conversion. For tandem reactions, the best yields were obtained when a solvent mixture of PhMe/DMF (1:1) was used. Thiazolidinone was prepared in 68% yield using a stepwise sequence and in 82% yield under tandem conditions. (Saiz C. *et al.,* **2009**)



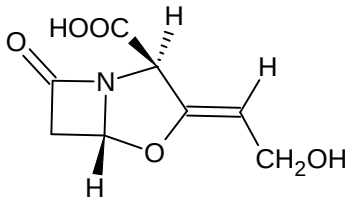
f) There are also several synthetic protocols for the synthesis of 4-thiazolidinones using catalysts like N, N'-dicyclohexylcarbodiimide (DCC), O-(benzotriazol-yl)-N,N,N',N'-tetramethyluronium hexafluoro phosphate (HBTU), ferrite, sodium sulfate and activated fly ash. The use of microwave heating, solid phase and polymer supported systems to run the cyclocondensation leading to 2,3-disubstitiuted 4-thiazolidinones have also been reported. (Umesh. *et al.*, **2011**)



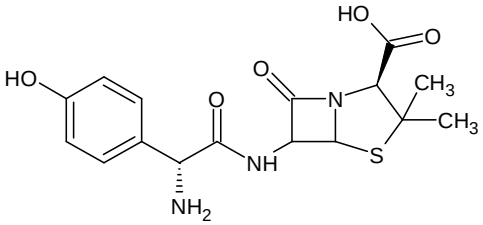
Penicillin (antibiotic)



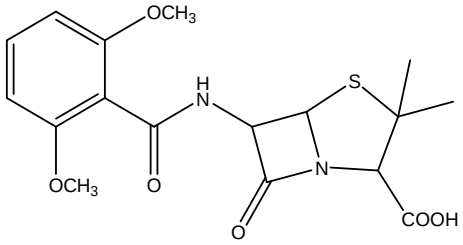
Cephalosporin (antibiotic)



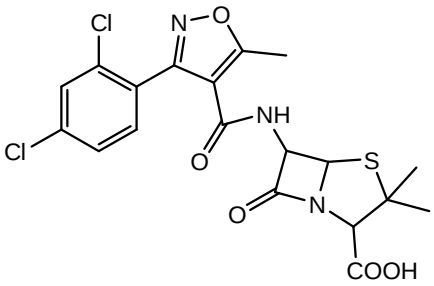
Clavulanic acid (antibiotic)



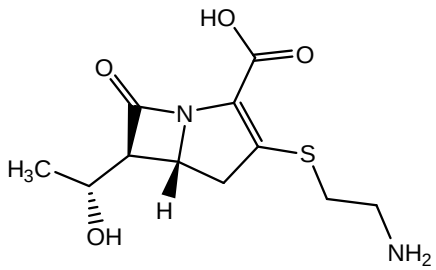
Amoxillicin (antibiotic)



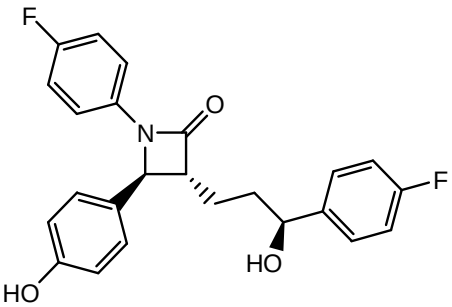
Methicillin (antibiotic)



Dicloxacillin (antibiotic)

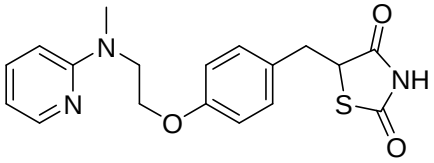


Thienamycin (antibiotic)

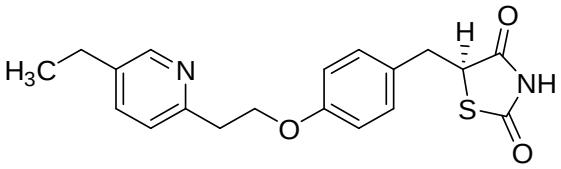


Ezetimibe (cholesterol inhibitor)

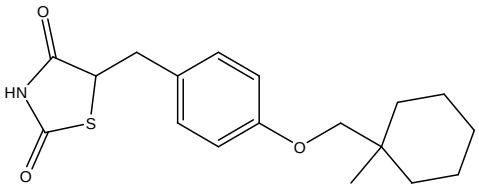
Fig-1: Compounds containing azetidinone nucleus



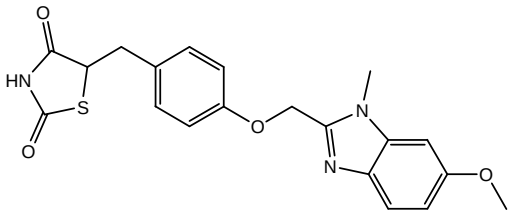
Rosiglitazone (antidiabetic)



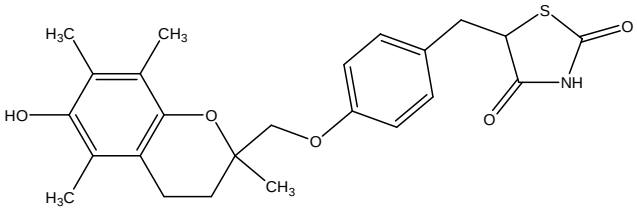
Pioglitazone (antidiabetic)



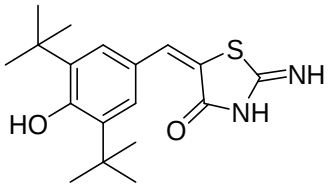
Ciglitazone (antidiabetic)



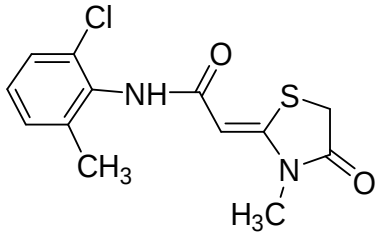
Rivoglitazon (antidiabetic)



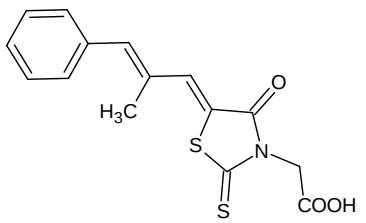
Troglitazone (antidiabetic)



Darbufelone (Anti-inflammatory)



Ralitoline (anticonvulsant)



Epalrestat (Aldose reductase inhibitor)

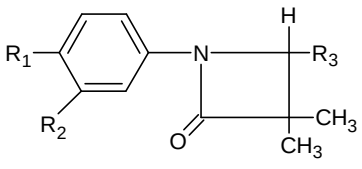
Fig-2: Compounds containing thiazolidinone nucleus

LITERATURE REVIEW

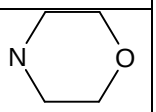
2. LITERATURE REVIEW

2.1. Literature review on azetidinone

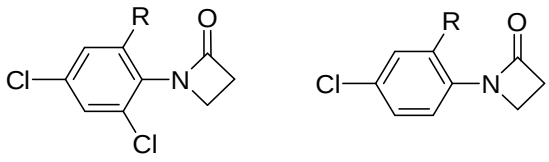
2.1.1) The preparation and antimicrobial activity of a series of β-amino-β-lactams was reported. These compounds were prepared from the 2+2 cycloaddition of β,β-disubstituted enamines with aryl isocyanates. Compounds underwent facile β-lactam ring fission between aminal carbon atom C_4 and the nitrogen N_1. The resulting formylactanilide derivatives were devoid of antibiotic activity. (Abdulla R.F. *et al*., **1975**)



(Fig: 3)

R_1	R_2	R_3
H	CF_3	NMe_2
CH_3	Cl	
Cl	H	H

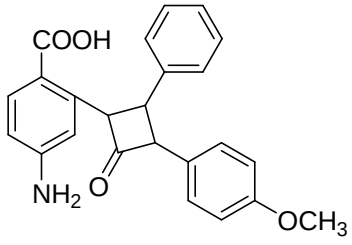
2.1.2) Some of the novel 2-azetidinone derivatives were synthesized and screened for anticonvulsant activity against electroshock and metrazole induced convulsions. The compounds showed moderate to good activity. (Peter. *et al*., **1972**)



(Fig: 4) R = Benzoyl

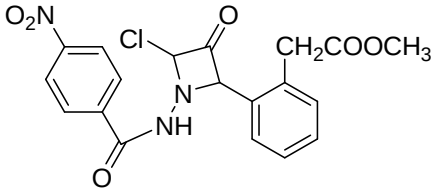
2.1.3) Synthesis of 4-amino-2-(3-(4-methoxyphenyl)-2-oxo-4-phenylcyclobutyl) benzoic acid was reported and screened for anti-inflammatory and antimicrobial activity. These were obtained by reacting p-nitroanthranilic acid and substituted aldehydes to get schiff bases which on treatment with chloroacetic acid obtained

different compounds. The compound showed good anti inflammatory and antimicrobial activity. (Udupi R.H. *et al*., **1996**)



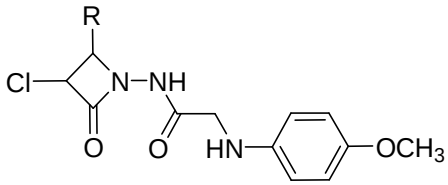
(Fig: 5)

2.1.4) Methyl-2-{2-[3-chloro-4-oxo-1-(4-nitrophenylcarbonylamino)azetidin-2yl]-phenoxy}-acetate was synthesized and screened for antibacterial activity. The compound showed good activity against *S. aureus* and *Salmonella typhi*. (Desai N.C. *et al.,* **2000**)



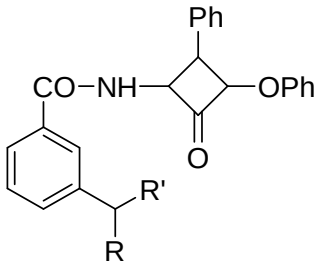
(Fig: 6)

2.1.5) A series of N-(2-aryl-3-chloro-4-oxoazetidin-1-yl)-2-(4-methoxyphenylamino) acetamides were synthesized from p-anisidine moiety. The structures of the newly synthesized compounds were confirmed by IR, ^{1}H NMR and mass spectroscopic analysis. The antibacterial and antifungal potential of the synthesized compounds were evaluated by the agar disc method. (Ishwar K. *et al*., **2007**)

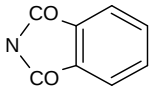
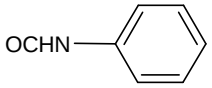
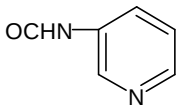
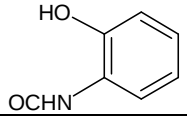


(Fig: 7)

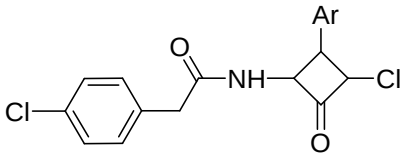
2.1.6) Synthesis, characterization and biological activity of 1,3,4-substituted 2-azetidinones was reported. All the compounds displayed high to moderate antiviral and antifungal activities. (Pandey V.K. *et al.*, **2005**)



(Fig: 8)

R	R’
H	
CH_2CH_3	
H	
C_6H_5	

2.1.7) N-(3-chloro-2-oxo-4-arylazetidin-1-yl)-2-(4-chlorophenyl) acetamides was prepared and evaluated for their antibacterial activity. The compounds showed good activity against *E.coli* and *S. aureus* in terms of minimum inhibitory concentration. Also QSAR studies for the compounds were done for structural and physicochemical parameters. (Desai N.C. *et al.*, **2008**)

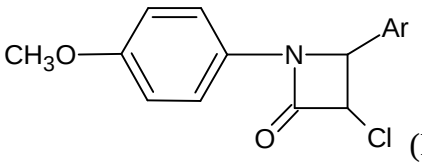


(Fig: 9)

Ar = 2-OH, 4-OH, 4-OCH_3, 4-Cl, 2-Cl, 4-CH_3

2.1.8) A series of 2-azetidinone derivatives were synthesized from p-anisidine and the synthesized compounds were confirmed by physical & spectral analysis. The compounds are evaluated for their antimicrobial properties. The activities are due to C=O, C-N, linkages in 2-azetidinones. All the compounds showed comparable

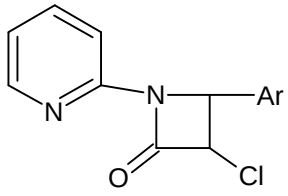
antimicrobial activities. Among these, 2-azetidinone having p-dimethyl amino phenyl at 4th position had shown good activity in all the species. (Jubie S. *et al.,* **2009**)



(Fig: 10)

Ar = phenyl, 2-chlorophenyl, 4-chlorophenyl, 3-chlorophenyl, p-dimethylaminophenyl

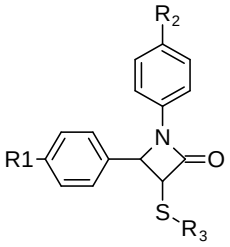
2.1.9) A series of 3-chloro 4-aryl N-pyridine -2-yl 2-azetidinone derivatives were prepared and characterized by IR, ^{1}H-NMR and Mass spectral studies. All the compounds were subjected to leptospirocidal study and the compounds showed significant to moderate activity. (Natarajan Ramalakshmishmi. *et al.,* **2009**)



(Fig: 11)

Ar = H, 2-OH, 4-OH, 4-OCH_3, 4-Cl, 2-Cl, 4-CH_3, 4-N $(CH_3)_2$, 3-NO_2, 4-NO_2

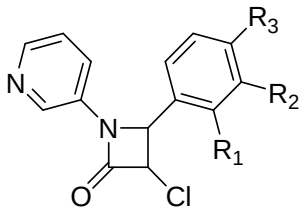
2.1.10) A series of 3-thiolated β-lactams were synthesized. All the compounds were characterized by spectral data and elemental analysis and were evaluated for their in vitro antibacterial and antifungal activities against various pathogenic strains. The preliminary screening results indicated that some of these compounds demonstrated moderate to very good antibacterial and antifungal activities. (Maaroof Zarei. *et al.,* **2011**)



(Fig: 12)

R^1	R^2	R^3
4-$NO_2C_6H_4$	4-$MeOC_6H_4$	C_6H_5
4-$NO_2C_6H_4$	4-ClC_6H_4	C_6H_5
4-$NO_2C_6H_4$	Me	C_6H_5
$C_6H_5CH_2$	4-$MeOC_6H_4$	C_6H_5
$C_6H_5CH_2$	4-ClC_6H_4	C_6H_5
$C_6H_5CH_2$	Me	C_6H_5

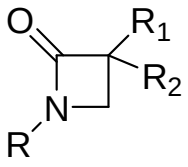
2.1.11) Some of the 2-azetidinone derivatives from 2-aminopyridine were synthesized. Structures of synthesized compounds were confirmed by physical and spectral analysis. The compounds are evaluated for their antimicrobial activities. The activities are due to cyclic CO-NH group in azetidinones. The following compounds showed significant activity against all the microbial strains. (Bijo Mathew. *et al*., **2010**)



(Fig: 13)

R_1	R_2	R_3
H	H	Cl
OH	H	H
H	OCH_3	H

2.1.12) A number of 2-azetidinone were synthesized and characterized. The compounds were found to possess good anticonvulsant activity in rats. The simultaneous presence of one aromatic and one aliphatic radical at R and R' was necessary for high activity, showed activity similar to that of Phenobarbital. (Maffi. *et al*., **1959**)

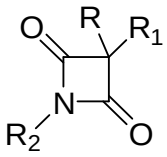


(Fig: 14)

R = H, CH_3, C_2H_5, C_6H5, p-NO_2 C_6H5, p-NH_2 C_6H5;

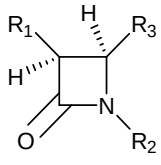
R_1 = Ph, $PhCH_2$; R_2 = H, CH_3, C_2H_5

2.1.13) A novel series of 2, 4-azetidinone compounds were synthesized and the compounds were tested for mycostatic, bacteriostatic and CNS activity. (Testa. *et al*., **1963**)



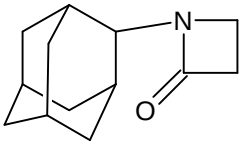
(Fig: 15) R = H; R_1 = cyclohexyl; R_2 = H

2.1.14) An efficient use of triphosgene, as an acid activator, for the synthesis of substituted 2-azetidinones via ketene-imine cycloaddition reaction using acids and imines was reported. (Krishnaswamy D. *et al*., **2002**)



(Fig: 16)

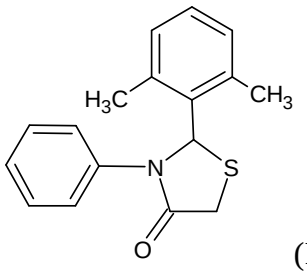
2.1.15) A novel adamantine derivative of 2-azetidinone was synthesized and tried its activity against influenza virus A-PR_8 and hepatitis virus MHV_3 and obtained encouraging results. (Levinine. *et al*., **1971**)



(Fig: 17)

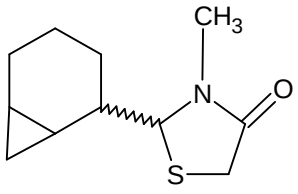
2.2. Literature review on thiazolidinone

2.2.1) A series of 2, 3-diaryl-1,3-thiazolidin-4-one derivatives were synthesized and characterized. Among them the compound 2-(2, 6-dimethylphenyl)-3-phenylthiazolidin-4-one was found to be most potent and reported as a new family of anti-viral agents acting as NNRTIs with minimal cytotoxicity. It was found that substitution by aryl group at nitrogen atom increases the cytotoxic activity of compound. (Monforte. *et al.,* **2001***)*



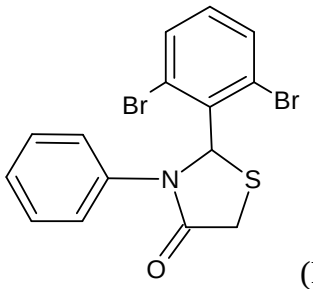
(Fig: 18)

2.2.2) 2-adamantyl-substituted thiazolidin-4- one derivative was synthesized and evaluated for its activity against HIV-1 (IIIB) and HIV-2(ROD) in CEM cell cultures, by taking Nevirapine as reference compound. Among them compound (19) was found to be more potent**.** Substitution by admantyl at second position was found to shown increased in cytotoxic activity. (Balzarini. *et al.,* **2007**)



(Fig: 19)

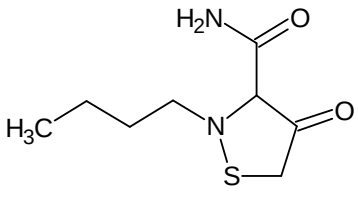
2.2.3) A series of 2-(2,6-dibromophenyl)-3-heteroaryl-1,3-thiazolidin-4-one derivatives were designed, synthesized and evaluated as selective HIV-1 RT enzyme inhibitors. The substitution of aryl group at nitro atom showed increased in cytotoxic activity. (Rawal. *et al.,* **2008**)



(Fig: 20)

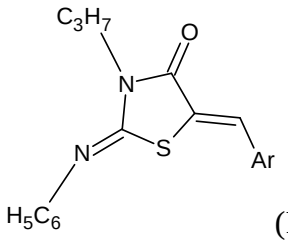
2.2.4) A series of 2-amyl-4-oxothiazolidin-3-yl amides were synthesized and these were evaluated for their ability to inhibit prostate cancer cell. Few potent compounds were detected, which were effective in killing prostate cancer cells with improved selectivity compared to serine amide phosphates. The Compound (21) was found to

be most potent. It was found that substitution at nitrogen atom by long alkyl chain causes increase in anticancer activity. (Gududuru. *et al*., **2004**)



(Fig: 21)

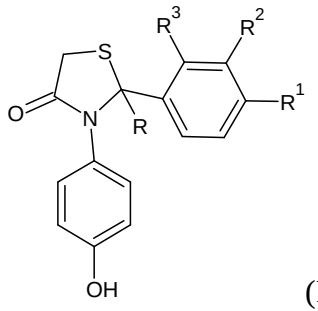
2.2.5) The synthesis and pharmacological activity of 5-arylidene-2-imino-4-thiazolidinone derivatives was reported. All the derivatives exhibited significant activity levels in models of acute inflammation such as carrageenan-induced paw and pleurisy edema in rats, by taking indomethacin as standard drug. In particular, 5-(3-methoxyphenylidene)-2-phenylimino-3-propyl-4-thiazolidinone displayed high levels of carrageenan-induced paw edema inhibition comparable to those of indomethacin. (Ottana. *et al*., **2005**)



(Fig: 22)

Ar= 3-$CH_3OC_6H_4$, 4-CH_3S C_6H_4, 4- $CH_3SO_2C_6H_4$, 4-$CH_3OC_6H_4$, 4-ClC_6H_4, 3, 4-$(CH_3O)_2C_6H_4$

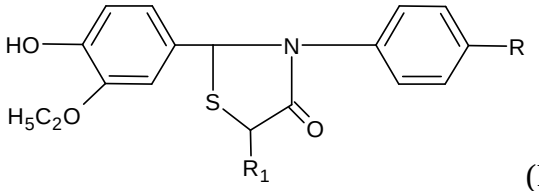
2.2.6) A new series of thiazolidine-4-one derivatives were synthesized and evaluated for anti-inflammatory, analgesic and anti-ulcer activity by carrageenan-induced paw edema test, acetic acid induced writhing method and pylorus ligation ulcer model respectively. All the compounds showed significant anti-inflammatory, analgesic and anti-ulcer activity at 100 mg/kg b.w. (Taranalli. *et al.,* **2009**)



R	R^1	R^2	R^3
H	H	H	H
H	OCH_3	H	H
H	CH_3	H	H
H	CH_3	CH_3	NH_3

(Fig: 23)

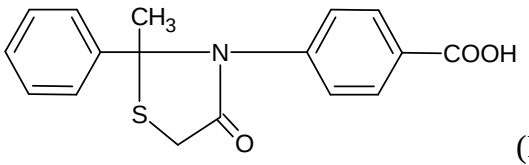
2.2.7) Synthesis and antifungal activity of 4-thiazolidinones derived from ethyl vanillin was reported. The synthesized compounds were characterized on the basis of elemental analysis and spectral studies and compounds were screened for antifungal activity. (Manrao M.R. *et al*., **1995**)



(Fig: 24)

R=H, Me, OMe, OEt, Cl

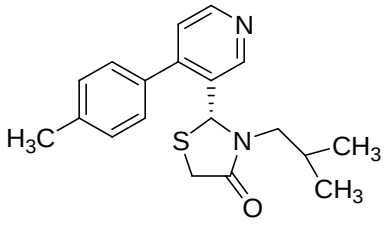
2.2.8) 2-Phenyl-2-methyl-3-aryl thiazolidin-4-ones had prepared by the addition of thioglycollic acid to substituted azomethines in benzene. The compound was reported for good antibacterial activity. (Shukla H.K. *et al*., **1981**)



(Fig: 25)

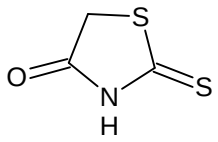
2.2.9) A series of new N-type calcium channel blockers derived from the 'hit' structures 2-(3-bromo-4-fluorophenyl)-3- (2-pyridin-2-ylethyl) thiazolidin-4-one and its 2-[4-(4-bromophenyl)pyridin-3-yl]-3-isobutyl analogue were synthesized. By SAR the compound have been identified as the most potent compounds in this series. These

compounds show promise as lead structures in the quest for clinically effective N-type blockers in the treatment of pain. (Lars. *et al.,* **2007**)



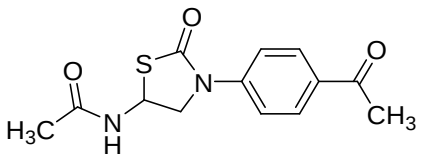
(Fig: 26)

2.2.10) 2-thioxo-4-thiazolidinone (rhodanine) derivatives are potential inhibitors of HIV integrase. These structures have been found to impart antitumor properties such as seen in rhodacyanine dyes. Moiety is an important structural scaffold for Integrase inhibitory activity. Such derivatives are suitable leads for antiviral and anticancer drug development. (Kavya Ramkumar. *et al*., **2010**)



(Fig: 27)

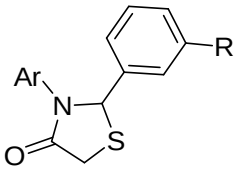
2.2.11) A novel synthesis of thiazolidine-2-thione and thiazolidine-2-one derivatives was described with the iodo-cyclosthiocarbamation reaction as the key step for the heterocyclic ring formation. This new method is applied to the synthesis of thiazolidinones as bioisosteric analogs of Linezoid and the antimicrobial properties of synthesized compounds were reported. (Viswajanani Sattigeri. *et al*., **2005**)



(Fig: 28)

2.2.12) A series of 1,3-thiazolidin-4-ones bearing various substituted diaryl ring at C-2 and N-3 positions were synthesized and evaluated for their anti-YFV activity.the compounds showed inhibitory effects on the replication of YFV in green monkey kidney (Vero) cells (ATCC CCL81), by means of a cytopathic effect reduction assay.

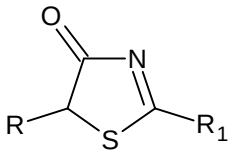
The synthesized compounds were found to be more effective than rabavirin and showed potent anti-YFV agent. (Dharmarajan. *et al.,* **2005**)



(Fig: 29)

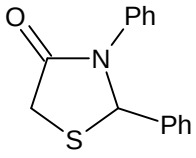
Code	Ar	R
DS1	4-F-C_6H_4	4-Cl
DS2	4-F- C_6H_4	2-Cl
DS3	4-F- C_6H_4	3-NO_2
DS4	2-C_5H_4N	4-Cl

2.2.13) A series of 4-thiazolidinone were synthesized and evaluated for their anti-inflammatory, analgesic, anticonvulsant and antimicrobial activity. All the compounds showed good biological activities. (Seema Mishra. *et al*., **1997**)



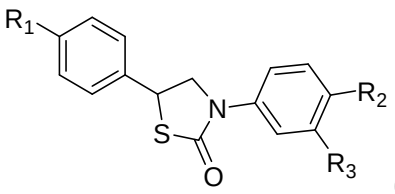
(Fig: 30)

2.2.14) Synthesis and anti-HIV activity of a series of 2,3-diaryl-1,3-thiazolid-4-ones were reported. The compounds exhibited moderate to good activity. (Barreca M.L. *et al*., **2001**)



(Fig: 31)

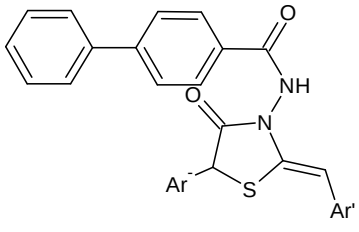
2.2.15) A series of 4-thiazolidinone derivatives were synthesized and the structure of the synthesized compounds were confirmed on the basis of IR and H-NMR. The synthesized compounds were screened for anti-inflammatory activity. Among the test compounds, III showed promising anti-inflammatory activity as compared to the control. (Vaidya. *et al.,* **2011)**



(Fig: 32)

Code	R_1	R_2	R_3
I	4-NO_2	4-F	3-Cl
II	4-NO_2	4-Br	H
III	4-SCH_3	4-OCH_3	H
IV	4-SCH_3	4-CF_3	H
V	4-OCH_3	H	3- CF_3

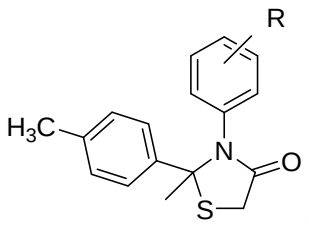
2.2.16) A novel derivatives of 4-thiazolidinone were prepared from biphenyl-4-carboxylic acid and evaluated for their *in vitro* antimicrobial activity against two gram negative strains (*E. coli and P. aeruginosa*) and two gram positive strains (*B. subtilis and S. aureus*) and fungal strain *C.albicans* and *A.* niger. The results revealed that all synthesized compounds have a significant biological activity against the tested microorganisms. (Aakash Deep. *et al*., **2011**)



(Fig: 33)

Ar= 3-BrC_6H_4; Ar'= 3-NO_2 C_6H_4, 3-BrC_6H_4

2.2.17) Some new series of 3-(substituted phenyl)-2-methyl-2-p-tolylthiazolidin-4-ones were prepared from 1-p-tolylethanone. The titled compounds were characterized on the basis of elemental analysis, IR, ^{1}H-NMR and ^{13}C-NMR spectral data. All the synthesized compounds were screened against four different bacterial strains *S. aureus, S. paratyphi-A, B. subtilis* and fungal strain *F. molaniforme* and *A. niger*. Some of them showed good antibacterial and antifungal activity compared to reference drugs used in the study. (Vikas A. Desai. *et al*., **2011**)

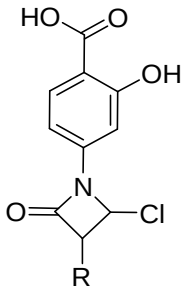


(Fig: 34)

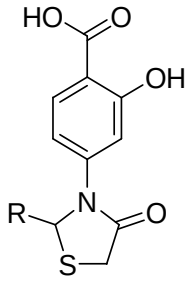
R= 4-Fluoro; 2, 4-difluoro; 3,5-difluoro; 2-methoxy; 3-Bromo

2.3. Literature review on azetidinone and thiazolidinone

2.3.1) A series of schiff's bases, 2-azetidinones, 4-thiazolidinones derivatives of the para aminosalicylic acid were synthesized. It was planned to employ the structure based computer aided drug designing (CADD) to schiff base, 2-azetidinone (Fig-35) and 4-thiazolidinone (Fig-36) of para aminosalicylic acid. Based on the data obtained from ligand-receptor binding studies, the novel molecules were synthesized and evaluated for their *in vitro* antimicrobial activity against Staphylococcus aureus, E.coli, candida albicans and Aspergillus nigar. The structure of synthesized compounds had established on the basis of their spectral (IR, ^{1}H NMR and mass) data. (Wadher S.J. *et al.*, **2009**)



(Fig: 35)

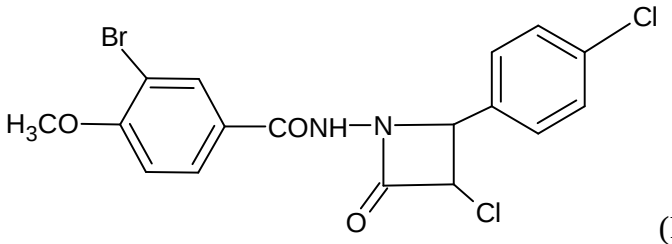


(Fig: 36)

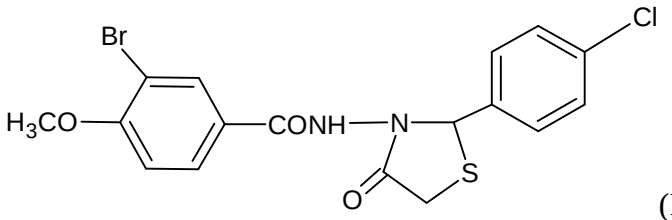
R= phenyl, 4-methoxyphenyl, 4-hydroxyphenyl

2.3.2) Synthesis of some azetidin-2-ones and thiazolidin-4-ones as potential antimicrobial agents were reported. 3-chloro-4-(substituted phenyl)-1-(3'-bromo-4'-methoxybenzamido)azetidin-2-ones (37) and 2-(substituted phenyl)-3-(3'-bromo-4'methoxy benzamido)thiazolidin -4-ones (38) were synthesized. The structure of the newly synthesized compounds was established by analytical spectral data. These

compounds were screened for their biological activity. (Havaldar Freddy H. *et al*., **2004**)



(Fig: 37)



(Fig: 38)

AIM

&

PLAN OF WORK

3. AIM AND PLAN OF WORK

Aim:

The chemistry of heterocyclic compounds continues to be an active field in the organic chemistry. Much attention has paid to the synthesis of heterocyclic compounds bearing nitrogen and sulphur containing ring systems like azetidin-2-ones and thiazolidin-4-ones. This may be mainly due to their wide range of biological activities. The synthesis of above class of compounds provides an outstanding case of modern drug development and also emphasizes the biological activity from structure modification to protype of drug molecules.

From the literature survey, it reveals substituent in the azetidinone and thiazolidinone may be varied, but the greatest difference in structure and properties is exerted by the group attached to the carbon atom in the 4^{th} position of azetidinone and the 2, 3, and 5-position of thiazolidin-4-none. Hence it shows, slight modification in structure can result in quantitative as well as qualitative changes in activity.

Since bulky substitution at all positions of 2-azetidinone and 4-thiazolidinone were reported and known to possess various biological activities such as antimicrobial, anti-inflammatory, analgesic activities, in the literature review.

So, in the present study, we decided to synthesize a new series of azetidin-2-one and thiazolidin-4-one compounds with simple substitution and to screen the newly synthesized compounds for their anti antimicrobial, analgesic and anti-inflammatory activity.

Plan of work:

- Synthesis of the following by appropriate method as per literature.
    - Schiff bases of p-amino benzoic acid
    - 2-Azetidinone derivatives
    - 4-Thiazolidinone derivatives
- Characterization of the newly synthesized compounds by

  Physical data such as
    - Solubility
    - Melting point
    - Thin layer chromatography

  Characterization and interpretation by
    - UV Visible Spectroscopy
    - IR Spectroscopy
    - ^{1}H-NMR Spectroscopy
    - Mass Spectroscopy
- To evaluate the antimicrobial activities for synthesized 2-azetidinone and 4-thiazolidinone derivatives.
- To screen the following biological activities only for the synthesized 4-thiazolidinone derivatives (SS_5 & SS_6):
    - Anti-inflammatory activity- Carrageenan induced paw edema method
    - Analgesic activity- Eddy's hot plate method

EXPERIMENTAL

4. EXPERIMENTAL

4.1 Materials

List of chemicals and drugs used:

S.No.	Chemicals/Drugs	Manufacturers
1	p-amino benzoic acid	Loba Chemie Pvt. Ltd., Mumbai
2	p-hydroxy benzaldehyde	Loba Chemie Pvt. Ltd., Mumbai
3	p-dimethyl benzaldehyde	Loba Chemie Pvt. Ltd., Mumbai
4	p-nitro benzaldehyde	Loba Chemie Pvt. Ltd., Mumbai
5.	Ethanol	Loba Chemie Pvt. Ltd., Mumbai
6.	Glacial acetic acid	Qualigens Fine Chemicals, Mumbai
7	Thioglycollic acid	Qualigens Fine Chemicals, Mumbai
8	Dioxane	Loba Chemie Pvt. Ltd., Mumbai
9.	Dimethyl formamide	Loba Chemie Pvt. Ltd., Mumbai
10.	Chloroacetyl chloride	Loba Chemie Pvt. Ltd., Mumbai
11.	Triethylamine	Qualigens Fine Chemicals, Mumbai
13.	Carrageenan Sodium	Sigma Chemical Co., USA
14.	Diclofenac sodium	German Remedies, Mumbai
15.	Benzene	Qualigens Fine Chemicals, Mumbai
16.	Acetone	Loba Chemie Pvt. Ltd., Mumbai
17.	Zinc chloride	Loba Chemie Pvt. Ltd., Mumbai
18.	Polyethylene glycol	Spectrochem Pvt. Ltd., Mumbai
19.	Ciprofloxacin	Hi-media Pvt. Ltd., Mumbai
20.	Ketoconazole	Hi-media Pvt. Ltd., Mumbai

List of instruments used:

S.no	Instruments	Manufacturer
1.	Melting point apparatus	Elico Ltd., Hyderabad
2.	Digital electronic balance	Shimadzu Ax - 200
3.	Heating Mandle	Ajay Thermo Electrics., Chennai
4.	UV-Visible spectrophotometer	Shimadzu 1601 UV-spectrophotometer, Japan
5.	^{1}H-NMR spectrophotometer	JEOL JNM-α 400 spectrometer
6.	FT-IR spectrophotometer	Shimadzu FTIR-8101 spectrophotometer
7.	Mass spectrophotometer	Jeol SX102/DA-6000 mass spectrometer
8.	Hot air oven	Precision Scientific Co., Chennai.
9.	Hot plate	Instrumental and Chemical Pvt. Ltd.,
10.	Plethysmometer	Inco rat paw Plethysmograph mercury model, Ambala, India.

Microorganisms:

The gram positive bacteria (*Staphylococcus aureus* ATCC 25923), gram negative bacteria *(Escherichia coli* ATCC 25922) and fungal strain (*Candida albicans* ATCC 2091) were collected from Microbial Resources Division, Kings Institute of Preventive Medicine, Guindy, Chennai. The agar medium was purchased from HI-media Laboratories Ltd., Mumbai, India.

Animals:

All the animals (albino mice and wistar albino rats, of either gender) were obtained from the Kings Institute of Preventive Medicine, Guindy, Chennai. Animals were housed in animal house in Adhiparasakthi College of Pharmacy in standard environmental conditions of temperature (25±20º C), humidity (55±10%) and light (12:12 hour light: dark cycle). The animals were fasted prior to dosing but water was

given *ad-libitum*. The anti-inflammatory and analgesic activity was carried out as per CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) guidelines after obtaining the approval from the Institutional Animal Ethical Committee.

ANALYTICAL TECHINQUE

Physical data

Melting point of the synthesized compounds were taken in the open capillary tubes.

Thin layer chromatography (TLC)

Purity of the compounds was checked by thin layer chromatography using silica gel G as stationary phase and benzene and acetone as mobile phase. The spot resolved were visualized by using iodine chamber.

UV spectra

The UV spectra of the synthesized compounds were recorded in the range of 200-400cm^{-1} and the absorbance maximum are reported in cm^{-1} at Adhiparasakthi College of Pharmacy.

Infrared spectra

The IR spectra were recorded on Fourier Transform IR spectrometer in the range of 400-4000cm^{-1} using KBr pellets and values of v_{max} are reported in cm^{-1} at Ideal Analytical and Research Institution, Puducherry.

^{1}H NMR spectra

^{1}H NMR spectra were recorded on DPX-400 MHz NMR spectrometer using DMSO-d_6 and chemical shift (δ) are reported in parts per million downfield from internal reference tetramethylsilane (TMS) at Sophisticated Analytical Instrumentation Facility, Panjab University, Chandigarh

Mass spectra

Mass spectra of the synthesized compounds were recorded at Sophisticated Analytical Instrumentation Facility, Indian Institute of Technology, Madras.

4.2 Methodology:

Monitoring of Synthetic Reaction Procedures:

Established synthetic procedures were employed for synthesis of compounds RS_1 to RS_{10} and the reactions were monitored by Thin Layer Chromatography (TLC) employing 6'' X 2'' plates coated with 0.25 mm thick layer of silica gel (pre-activation by heating at 110°C for one hour). Solvent systems of varying polarity ranging from benzene to benzene to acetone mixtures (9:1, 5:5, 8:2, 7:3, 6:4, and 4:6) were used to monitor the reactions. The plates were visualized in iodine chamber.

Purification Techniques:

Recrystallization: The crude products were recrystallized in appropriate solvent. Single solvent was used wherever possible and solvent mixtures were not used anywhere.

Authentication of Chemical Structures and Purity of Compounds:

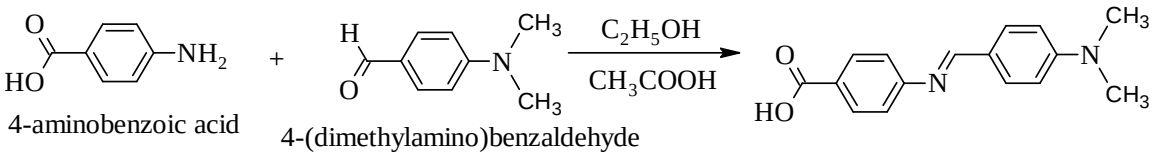
Chemical structure of products and their purity were ascertained by thin layer chromatography, UV-visible spectrometer, melting point and various spectral techniques including Fourier Transform Infra Red Spectroscopy, Nuclear Magnetic Resonance Spectroscopy, Mass Spectroscopy and Ultra Violet Spectrophotometer.

SYNTHESIS OF COMPOUNDS

4.3. SYNTHESIS OF COMPOUNDS

4.3.1. Synthesis of 4-[4-(dimethylamino benzylidene) amino] benzoic acid (SB_1)

1.23 g (0.01 mol) of p-amino benzoic acid was dissolved in 30 ml ethanol containing few drops of glacial acetic acid. 1.49 g (0.01mol) of p-dimethylamino benzaldehyde was added to the reaction mixture and stirred well. The reaction mixture was refluxed for 3 to 5 h, cooled and then poured into crushed ice .The solid obtained was filtered, washed with water and recrystallized from ethanol. (Jubie S. *et al*., **2009**)

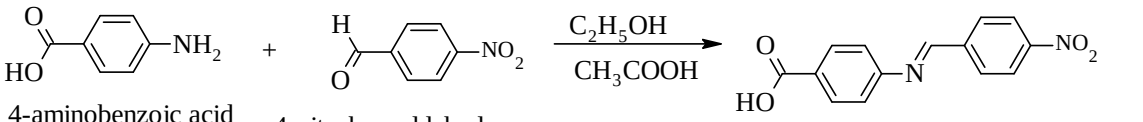


4-aminobenzoic acid 4-(dimethylamino)benzaldehyde

Scheme-1: Synthesis of 4-[4-(dimethylamino benzylidene) amino] benzoic acid

4.3.2. Synthesis of 4-[4-(nitrobenzylidene)amino]benzoic acid (SB_2)

1.23 g (0.01 mol) of p-amino benzoic acid was dissolved in 30 ml ethanol containing few drops of glacial acetic acid. 1.15 g (0.01 mol) of p-nitro benzaldehyde was added to the reaction mixture and stirred well. The reaction mixture was refluxed for 3 to 5 h, cooled and then poured into crushed ice .The solid obtained was filtered, washed with water and recrystallized from ethanol.

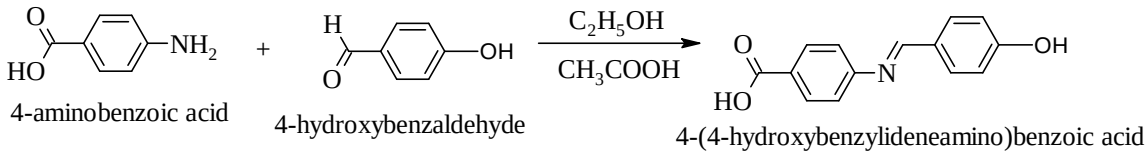


4-aminobenzoic acid 4-nitrobenzaldehyde 4-(4-nitrobenzylideneamino)benzoic acid

Scheme-2: Synthesis of 4-[4-(nitrobenzylidene) amino] benzoic acid

4.3.3. Synthesis of 4-[4-(hydroxybenzylidene) amino] benzoic acid (SB_3)

1.23 g (0.01 mol) of p-amino benzoic acid was dissolved in 30 ml ethanol containing few drops of glacial acetic acid. 1.22 g (0.01 mol) of p-hydroxybenzaldehyde was added to the reaction mixture and stirred well. The reaction mixture was refluxed for 3 to 5 h, cooled and then poured into crushed ice .The solid obtained was filtered, washed with water and recrystallized from ethanol.

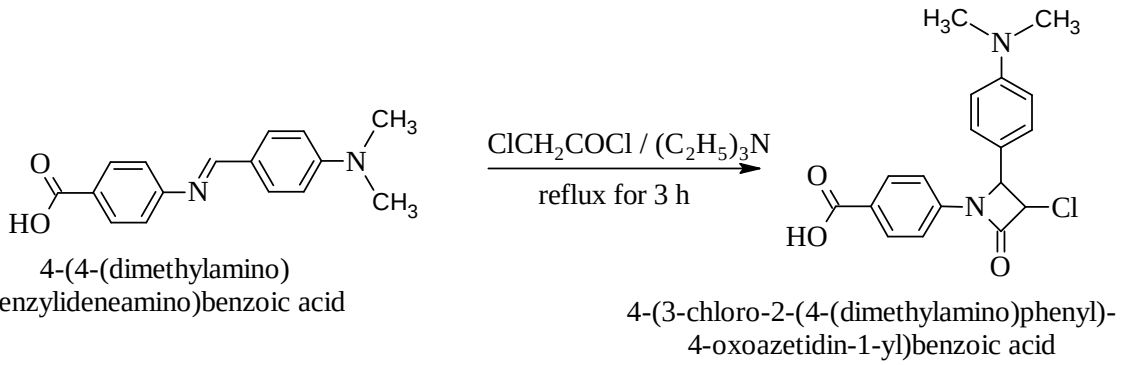


4-aminobenzoic acid — 4-hydroxybenzaldehyde — 4-(4-hydroxybenzylideneamino)benzoic acid

Scheme-3: Synthesis of 4-[4-(hydroxybenzylidene) amino] benzoic acid

4.3.4. Synthesis of 4-(3-chloro-2-(4-dimethylaminophenyl)-4-oxoazetidin-1-yl) benzoic acid (SS_1)

To a solution of appropriate amount of 4-[(4(dimethylamino)benzylidene)amino] benzoic acid (0.01 mol) in Dioxane (15 ml), triethyl amine (0.02 mol) was added. To this, a solution of chloroacetyl chloride (0.02 mol) was added in portion wise with vigorous shaking at room temperature for 20 min and heated under reflux for an 3 h and the content was kept at room temperature for 48 h. The azetidinone compounds, which separated on dilution with ice cold water was collected, dried and recrystallized from ethanol. (Raga Basawaraj. *et al.*, **2010**)



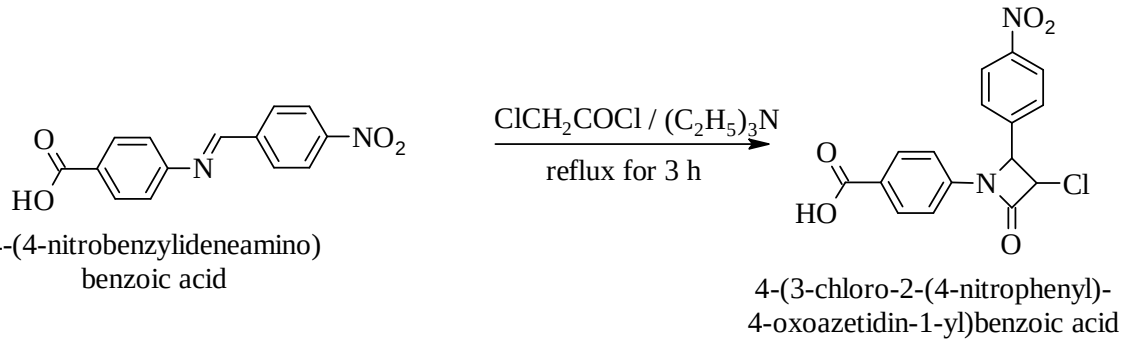
4-(4-(dimethylamino) benzylideneamino)benzoic acid

4-(3-chloro-2-(4-(dimethylamino)phenyl)-4-oxoazetidin-1-yl)benzoic acid

Scheme-4: Synthesis of 4-(3-chloro-2-(4-dimethylaminophenyl)-4-oxoazetidin-1-yl) benzoic acid

4.3.5. Synthesis of 4-(3-chloro-2-(4-nitrophenyl)-4-oxoazetidin-1-yl) benzoic acid (SS_2)

To a solution of appropriate amount of 4-[4-(nitrobenzylidene)amino]benzoic acid (0.01 mol) in Dioxane (15 ml), triethyl amine (0.02 mol) was added. To this a solution of chloroacetyl chloride (0.02 mol) was added in portion wise with vigorous shaking at room temperature for 20 min and heated under reflux for an 3 h and content was kept at room temperature for 48 h. The azetidinone compounds, which separated on dilution with ice cold water was collected, dried and recrystallized from ethanol.



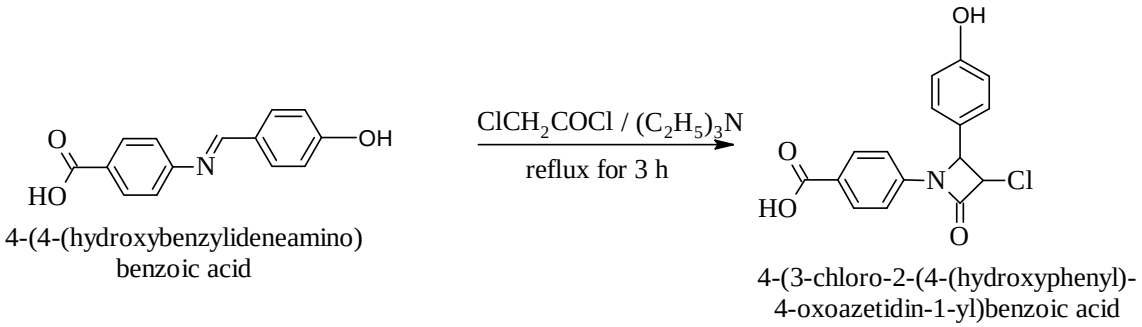
4-(4-nitrobenzylideneamino) benzoic acid

4-(3-chloro-2-(4-nitrophenyl)-4-oxoazetidin-1-yl)benzoic acid

Scheme-5: Synthesis of 4-(3-chloro-2-(4-nitrophenyl)-4-oxoazetidin-1-yl) benzoic acid

4.3.6. Synthesis of 4-(3-chloro-2-(4-hydroxyphenyl)-4-oxoazetidin-1-yl) benzoic acid (SS_3)

To a solution of appropriate amount of 4-[4-(hydroxybenzylidene) amino]benzoic acid (0.01 mol) in Dioxane (15 ml), triethyl amine (0.02 mol) was added. To this a solution of chloroacetyl chloride(0.02 mol) was added in portion wise with vigorous shaking at room temperature for 20 min and heated under reflux for an 3 h and content was kept at room temperature for 48 h. The azetidinone compounds, which separated on dilution with ice cold water was collected, dried and recrystallized from ethanol.



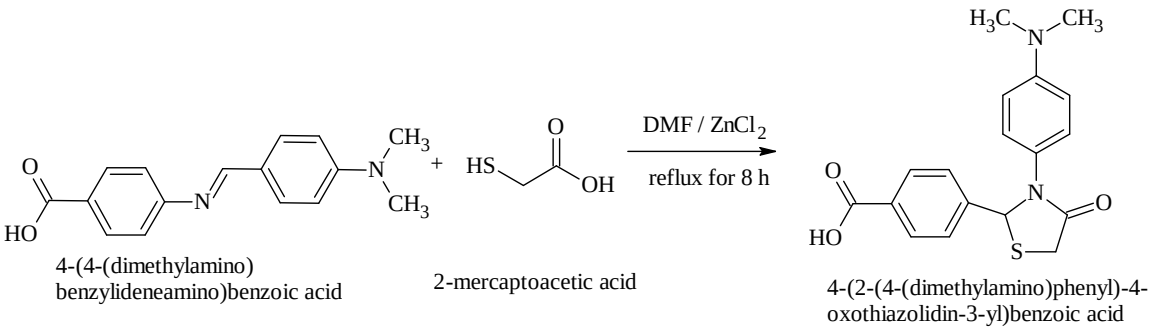
4-(4-(hydroxybenzylideneamino) benzoic acid

4-(3-chloro-2-(4-(hydroxyphenyl)-4-oxoazetidin-1-yl)benzoic acid

Scheme-6: Synthesis of 4-(3-chloro-2-(4-hydroxyphenyl)-4-oxoazetidin-1-yl) benzoic acid

4.3.7. Synthesis of 4-(2-[4-(dimethylamino)phenyl]-4-oxo-1,3-thiazolidin-3yl)-benzoic acid (SS_4)

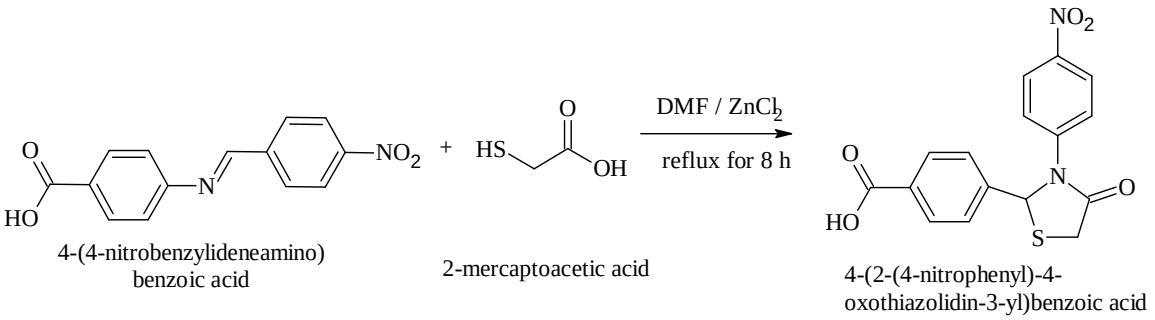
A mixture of 3.42 g (0.01 mol) of 4-[(4-(dimethylamino)benzylidene)amino]benzoic acid (0.01 mol) and mercaptoacetic acid (0.012 mol) was dissolved in 25 ml of dimethyl formamide. To this a pinch of anhydrous zinc chloride was added and then refluxed for 8 h. The reaction mixture was allowed to cool and poured into ice-cold water. The resulting solid was filtered, washed several times with water and recrystallized from ethanol. (Deepak Pareek A. *et al*., **2011**)



Scheme-7: Synthesis of 4-{2-[4-(dimethylamino)phenyl]-4-oxo-1,3-thiazolidin-3-yl}benzoic acid

4.3.8. Synthesis of 4-{2-[4-nitrophenyl]-4-oxo-1,3-thiazolidin-3-yl}benzoic acid (SS_5)

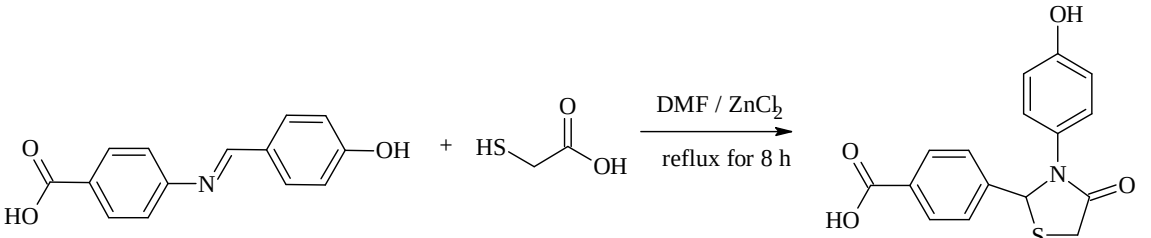
A mixture of 3.44 g (0.01 mol) of 4-[4-(nitrobenzylidene)amino]benzoic acid (0.01 mol) and mercaptoacetic acid (0.012 mol) was dissolved in 25 ml of dimethyl formamide. To this a pinch of anhydrous zinc chloride was added and then refluxed for 8 h. The reaction mixture was allowed to cool and poured into ice-cold water. The resulting solid was filtered, washed several times with water and recrystallized from ethanol.



Scheme-8: Synthesis of 4-{2-[4-nitrophenyl]-4-oxo-1, 3-thiazolidin-3-yl} benzoic acid

4.3.9. Synthesis of 4-{2-[4-hydroxyphenyl]-4-oxo-1, 3-thiazolidin-3-yl}benzoic acid (SS_6)

A mixture of 3.14 g (0.01 mol) of 4-[4-(hydroxybenzylidene) amino] benzoic acid (0.01 mol) and mercaptoacetic acid (0.012 mol) was dissolved in 25 ml of dimethyl formamide. To this a pinch of anhydrous zinc chloride was added and then refluxed for 8 h. The reaction mixture was allowed to cool and poured into ice-cold water. The resulting solid was filtered, washed several times with water and recrystallized from ethanol.



4-(4-hydroxybenzylideneamino) benzoic acid

2-mercaptoacetic acid

4-(2-(4-hydroxyphenyl)-4-oxothiazolidin-3-yl)benzoic acid

Scheme-9: Synthesis of 4-{2-[4-hydroxyphenyl]-4-oxo-1, 3-thiazolidin-3-yl} benzoic acid

BIOLOGICAL EVALUATION

5. BIOLOGICAL SCREENING

5.1. Evaluation of antimicrobial activity

The antimicrobial screening of the synthesized compounds (SS_1-SS_6) were carried out by determining the zone of inhibition using disc diffusion method. The synthesized compounds were dissolved in DMSO and sterilized by filtering through 0.45 µm millipore filter. Final inoculums of 100 µl suspension containing 10^8 CFU/ ml of each bacterium and fungus used. Nutrient agar (anti bacterial activity) and sabouraud dextrose agar medium (antifungal activity) was prepared and sterilized by an autoclave (121 º C and 15 lbs for 20 min) and transferred to previously sterilized petridishes (9 cm in diameter). After solidification, petriplates were inoculated with bacterial organisms in sterile nutrient agar medium at 45^o C, and fungal organism in sterile sabouraud dextrose agar medium at 45^o C in aseptic condition. Sterile whatmann filter paper discs (previously sterilized in U.V. lamp) were impregnated with synthesized compounds at a concentration of 25,100 µg/discs was placed in the organism-impregnated petri plates under sterile condition. The plates were left for 30 min to allow the diffusion of compounds at room temperature. Antibiotic discs of ciprofloxacin (100 µg/disc) and ketaconazole (100 µg/disc) were used as positive control, while DMSO used as negative control. Then the plates were incubated for 24 h at 37 ± 1^o C for antibacterial activity and 48 h at 37 ± 1^o C for antifungal activity. The zone of inhibition was calculated by measuring the minimum dimension of the zone of no microbial growth around the each disc. (Jubie S. *et al.*, **2009**)

5.1.a. Acute oral toxicity studies

The acute oral toxicity studies of synthesized compounds (SS_5 & SS_6) were performed to study the acute toxic effects and to determine minimum lethal dose of the drug extracts LD_{50}. Swiss albino mice of either sex weighing 20-25 g were used for the study. The animals were fasted overnight prior to the experiment and were then grouped (3 mice per group). They were treated intraperitoneally with different doses of the test compounds (5, 50, 300 mg/kg). After administration of the compounds, animals were observed continuously for the first three hours for any toxic manifestation. The animals were then observed for 48 h. Further the animals were under investigation up to a period of one week. (Hussain K. *et al*., **2005**)

5.2. Evaluation of Anti-inflammatory activity

The anti-inflammatory activity of the synthesized derivative (SS_5 & SS_6) was evaluated by carrageenan induced paw edema method.

Wister rats of either sex (150-200 g) were randomly selected and the animals were divided into control, standard and test groups, each consisting of three animals. The first group was treated with 1% polyethylene glycol which served as control, second group was administered with a dose of 20 mg/kg diclofenac sodium intraperitoneally which served as standard and other groups III and IV were treated with 50 mg/kg of SS_5 and SS_6 respectively by intraperitoneal route. After 30 min, the rats were injected with 0.1 ml of carrageenan (1% w/v) to the sub plantar region of left paw of the rats. The volume of paw was measured using mercury displacement technique with the help of plethysmograph both in control and animals treated with standard and test compounds at 0, 1, 2 and 3 h after injection of carrageenan.

The percentage inhibition of oedema was calculated by using formula,

$$\text{Percentage reduction} = \frac{Vo - Vt}{Vo} \times 100$$

Where Vt = mean paw volume of the test drug

Vo = mean paw volume of the control

From the data obtained, the mean odema volume and percentage reduction in odema was calculated. (Gurupadyya. *et al*., **2008**)

5.3. Evaluation of analgesic activity

The analgesic activity of the synthesized derivatives (SS_5 & SS_6) was evaluated by Eddy's hot plate method. Albino mice of either sex (20-25 g) were randomly selected and the animals were divided into control, standard and test groups, each consisting of three animals. The first group was treated with 1% polyethylene glycol suspension which served as control, second group was administered with pentazocine in the dose of 20 mg/kg , intraperitoneally which served as standard and other groups were treated intraperitoneally with 50 mg/kg of test compounds in polyethylene glycol. The animals were placed on hot plate and the pain was induced by 55^{o} C heat and the reaction time that is paw licking or jump response which ever appears was recorded at 0, 30, 60, 90 and 180 min after the drug administration. As soon as response was shown the animals are withdrawn from the plate immediately. The cut off period of 15sec was maintained to avoid damage to the paw. (Srivastava S.K. *et al*., **2006**)

RESULTS
&
DISCUSSION

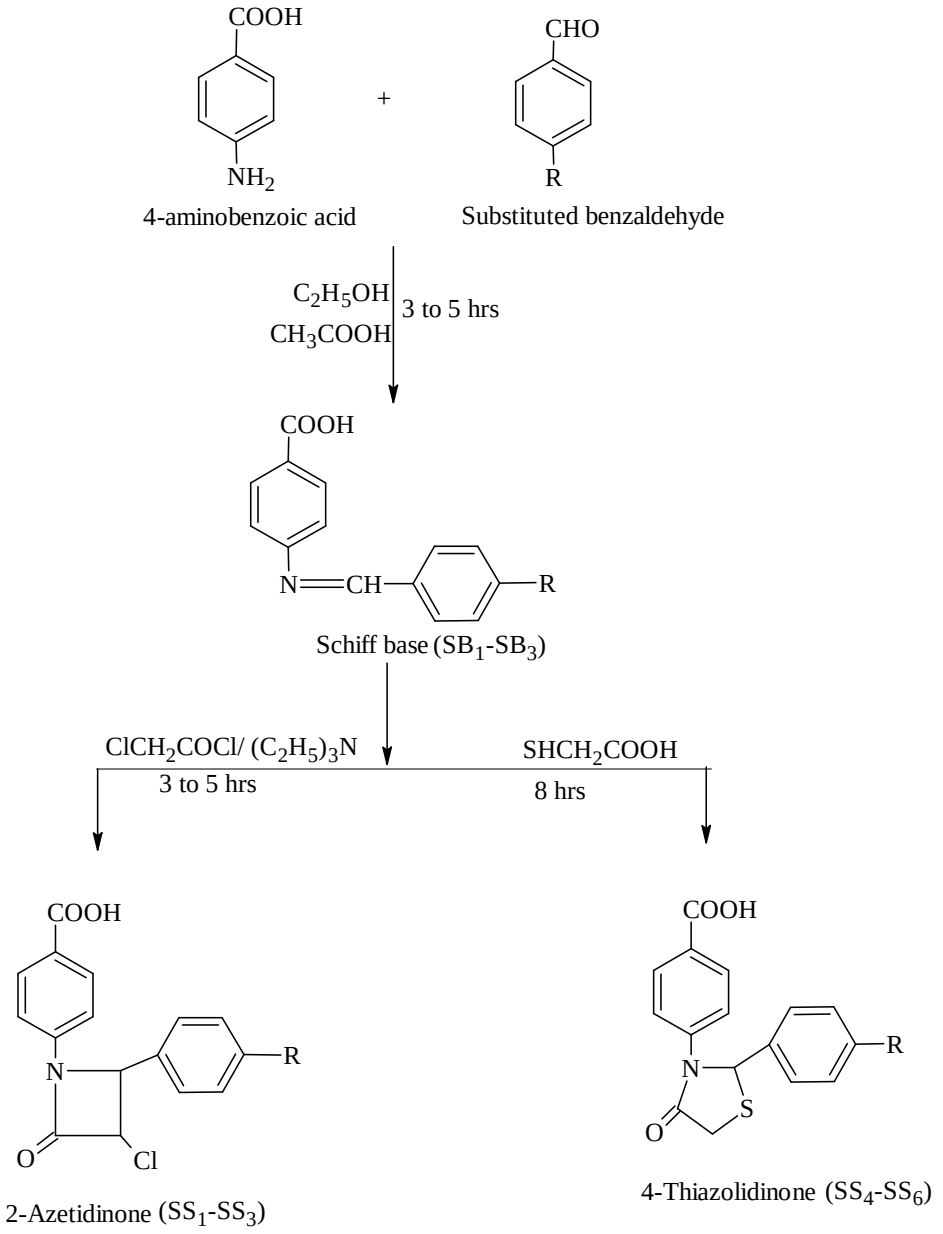
6. RESULTS AND DISCUSSION

6.1. Synthetic Scheme

2-Azetidinones and 4-thiazolidinones are the most common and important groups among the small ring heterocyclic compounds. Many methods had been reported in the literature for the synthesis of 2-azetidinone such as ketene-imine cycloaddition, wasserman cyclization, acid chloride addition reaction and also by using microwave irradiation method. Among these Staudinger ketene-imine cyclization is the most common method which involves the reaction of Schiff base (imines) with acid chloride in the presence of tertiary base. (Mrunmayee Toraskar. *et al.,* **2010**)

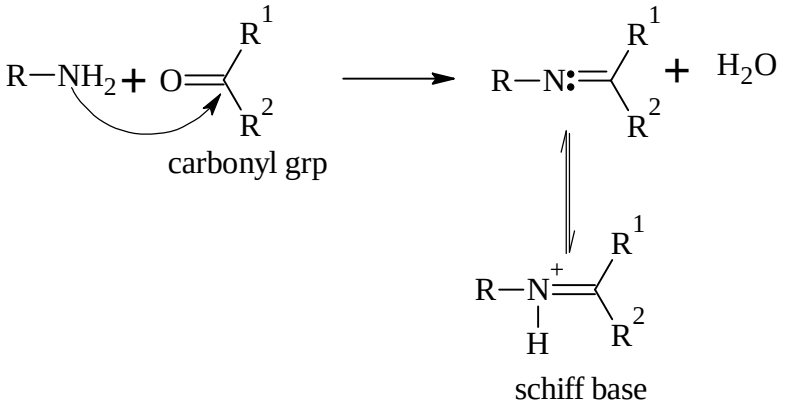
Several synthetic protocols for 4-thiazolidinones are reported in the literature such as conventional one pot, two pot, microwave as well as combinatorial synthesis. The most common method used was the reaction between Schiff bases (imines) with mercaptoacetic acid to form 4-thiazolidinones. (Mulay Abhinit. *et al.,* **2009**)

In this present study, a series of 2-azetidinone and 4-thiazolidinone were synthesized by the following strategy: initially Schiff bases (SB_1-SB_3) was synthesized by the condensation of p-amino benzoic acid with three different aromatic aldehydes. Cyclo-condensation of Schiff bases with chloroacetyl chloride in the presence of tri ethylamine resulted in the formation of corresponding azetidinone analogues (SS_1-SS_3). Similarly cyclocondensation of Schiff base with mercapto acetic acid yielded the corresponding thiazolidinone analogues (SS_4-SS_6).

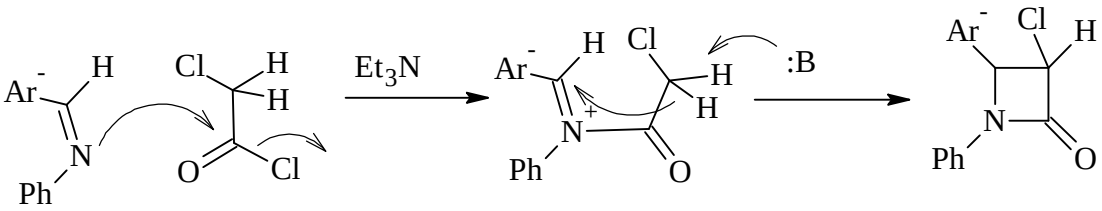


Code	**R**
SB_1,SS_1,SS_4	$N(CH_3)_2$
SB_2,SS_2,SS_5	NO_2
SB_3,SS_3,SS_6	OH

Scheme-10: General synthetic route for 2-azetidinone and 4-thiazolidinone derivatives

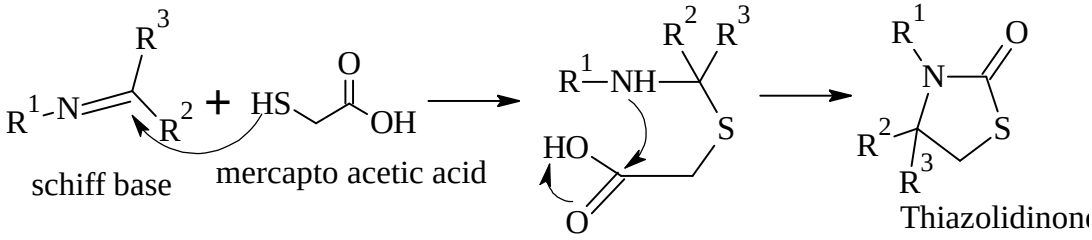


Scheme-11: Mechanism for formation of Schiff base



Scheme-12: Mechanism for formation of azetidinone

Both the ketene and the imine are molecules that can act as either nucleophiles or electrophiles. In the first step, the imine adds to the ketene as a nucleophile. The subsequent cycloaddition delivers the azetidin-2-one. (Verma M. *et al*., **1974**)



Scheme-13: Mechanism for formation of thiazolidinone

The nucleophilic attack of the mercapto acetic acid anion on carbon of azomethine (Schiff base) forms an uncyclized intermediate. The subsequent cycloaddition delivers the thiazolidin-4-one. (Verma M. *et al*., **1974**)

INTERPRETATION OF SYNTHESIZED COMPOUND

6.2 Interpretation of spectral data of synthesized compounds

6.2.1 Spectral analysis of 4-[4-(dimethylaminobenzylidene)amino]benzoic acid (SB_1)

UV: (Fig-39)

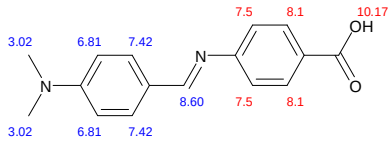
λ_{max} (MeOH) 341.0 (ε_{max} 0.3896)

λ_{max} (MeOH) 267.0 (ε_{max} 0.9121)

IR (KBr): (Fig-40)

Wavelength (cm^{-1})	**Assignment**
2917.19	Aromatic C-H (stretching)
1679.83	C=N (stretching)
1585.05	Aromatic C=C (stretching)
1417.45	C-O-H (bending)
1434.40	N-CH_3 (stretching)
1286.58	C-O (stretching)
819.47	Aromatic C-H (bending)

NMR (DMSO-d_6): (Fig- 41&42)



(8 aromatic protons, 6 methyl protons, 1 proton on Schiff base and 1 proton on carboxylic acid)

Δ	**Assignment**
10.17	(1H, s, Ar-COOH)
8.60	(1H, s, CH=N)
8.1-7.50	(4H, m, Ar-H of benzoic acid)
7.42-6.81	(4H, m, Ar-H of dimethylamino phenyl group)
3.02	(6H, s, $N(CH_3)_2$)

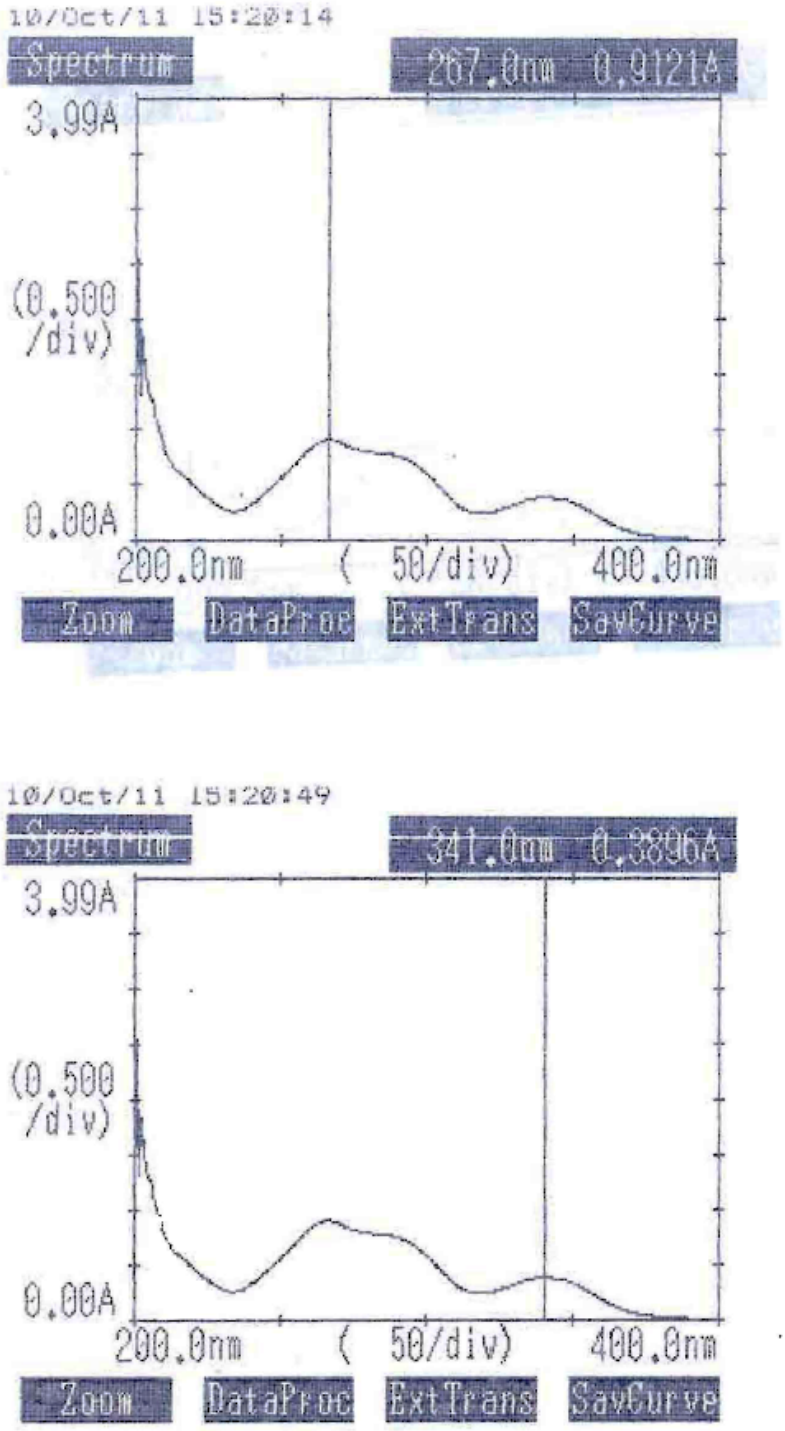


Fig- 39 : UV Spectrum of compound SB_1

IDEAL ANALYTICAL AND RESEARCH INSTITUTION, PUDUCHERRY.

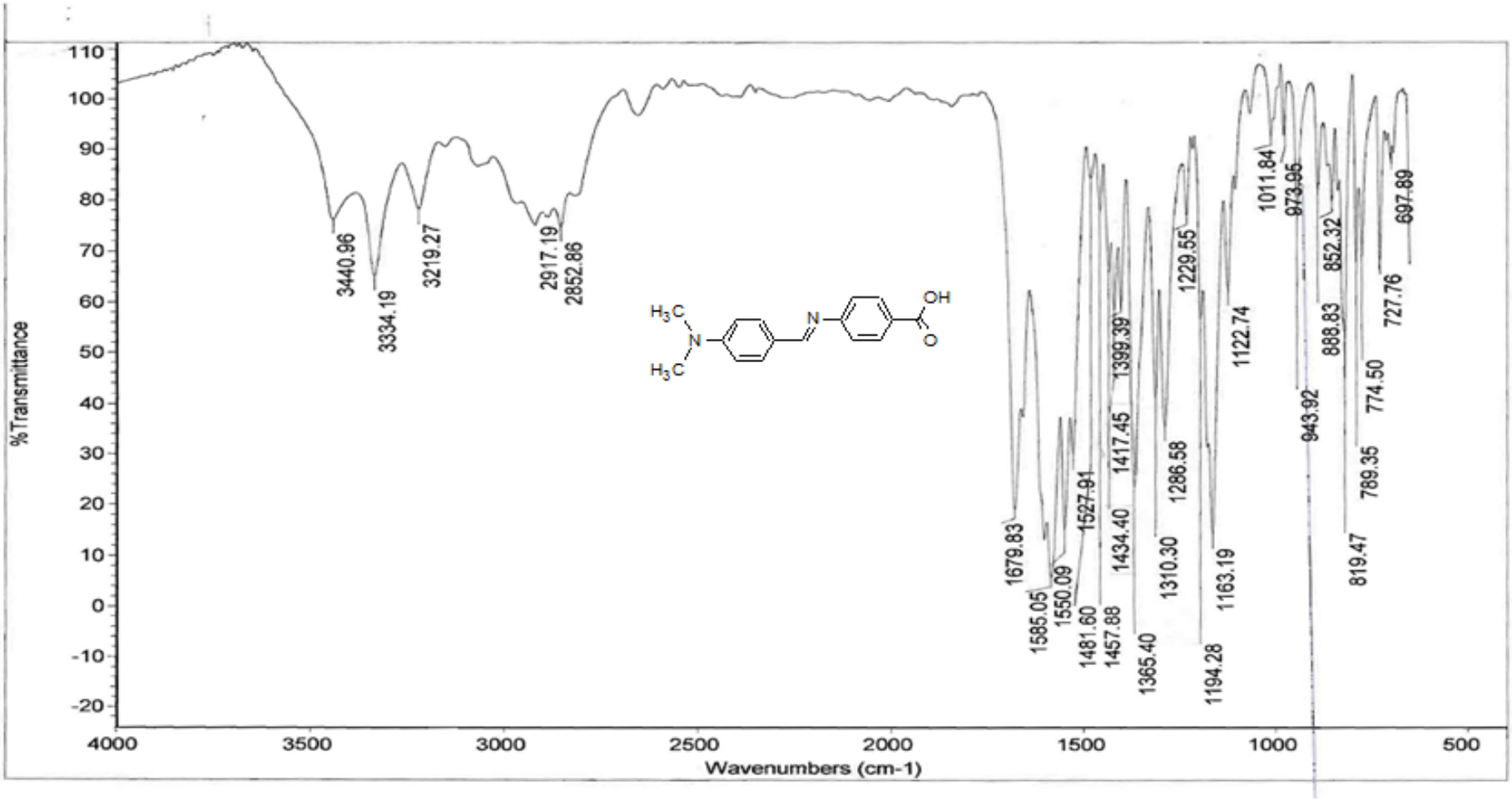


Fig- 40: IR Spectrum of compound SB_1

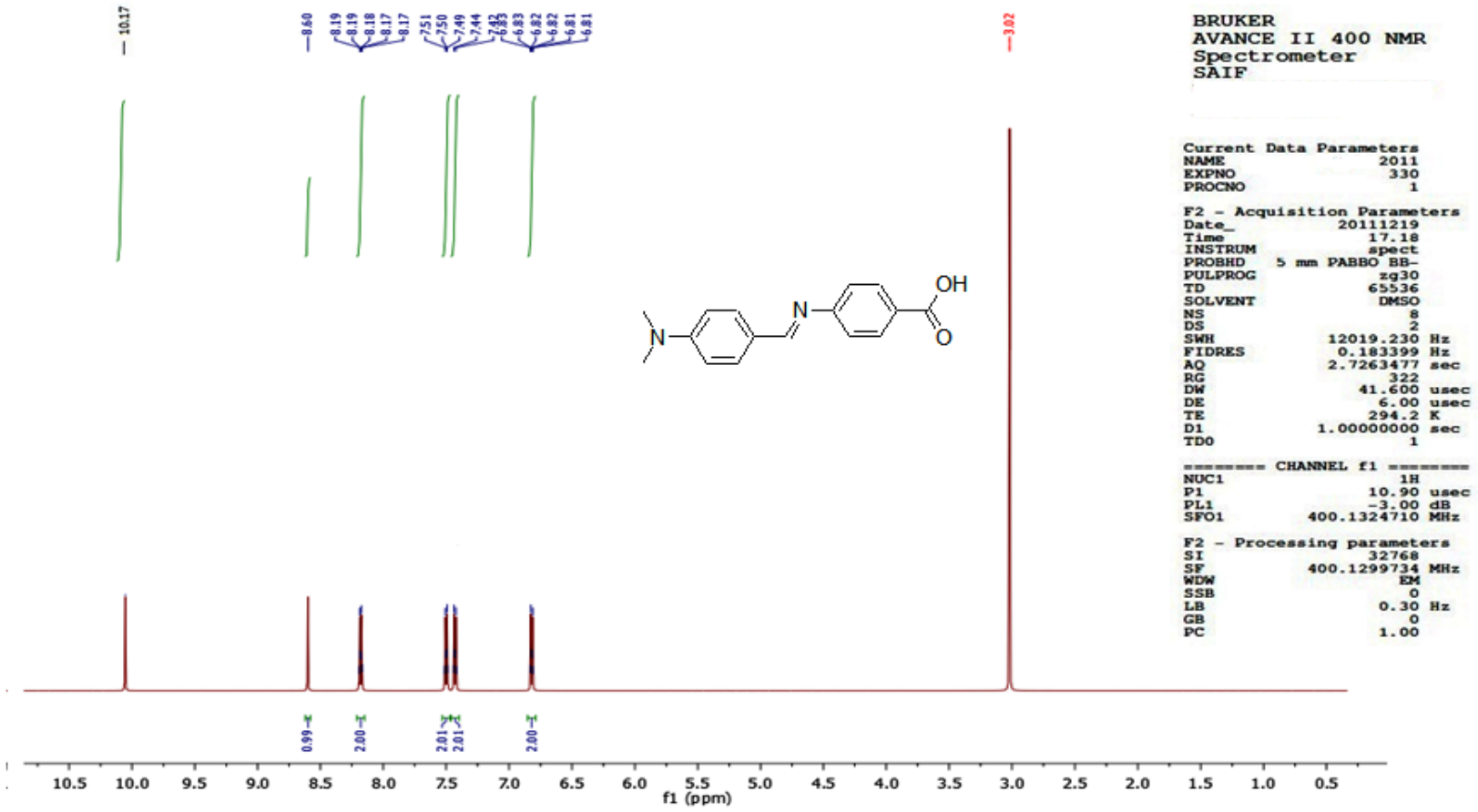


Fig- 41: ^{1}H-NMR Spectrum of the compound SB_1

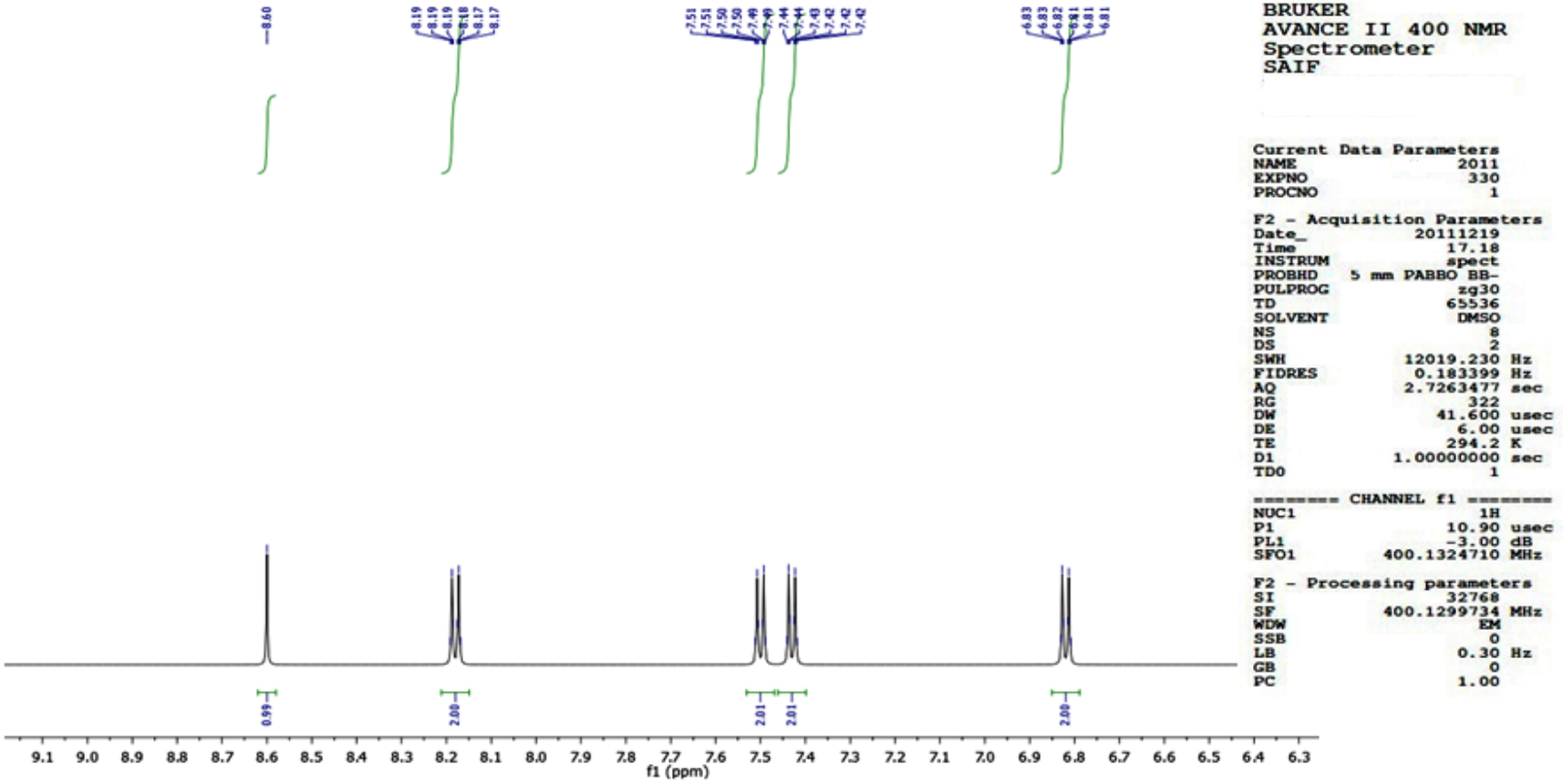


Fig- 42:^{1}H-NMR Spectrum of the compound SB_1(Zoom View)

6.2.2 Spectral analysis of 4-[4-(nitrobenzylidene) amino] benzoic acid (SB_2)

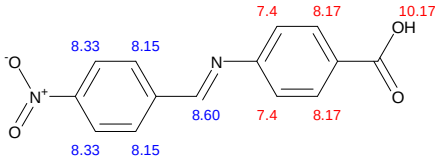
UV: (Fig-43)

λ_{max} (MeOH) 275.0 (ε_{max} 1.0647)

IR (KBr): (Fig-44)

Wavelength (cm^{-1})	Assignment
3074.08	Aromatic C-H (stretching)
1681.92	C=N (stretching)
1589.52	Aromatic C=C (stretching)
1516.59	Aromatic NO_2 asymmentric (stretching)
1427.20	C-O-H (bending)
1343.43	Aromatic NO_2 symmentric (stretching)
1295.32	C-O (stretching)
832.22	Aromatic C-H (bending)

NMR (DMSO-d_6): (Fig-45&46)



(8 aromatic protons, 1 proton on Schiff base and 1 proton on carboxylic acid)

δ	Assignment
10.17	(1H, s, Ar-COOH)
8.60	(1H, s, CH=N)
8.1-7.4	(8H, m, Ar-H)

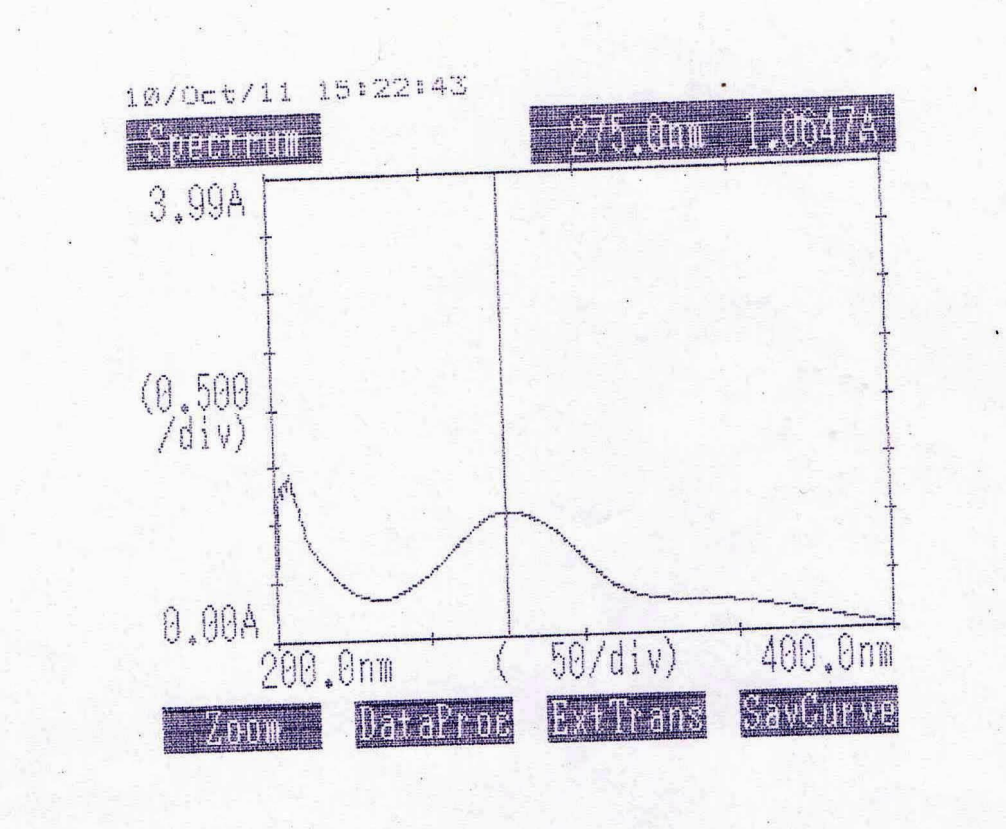


Fig-43 : UV Spectrum of compound SB_2

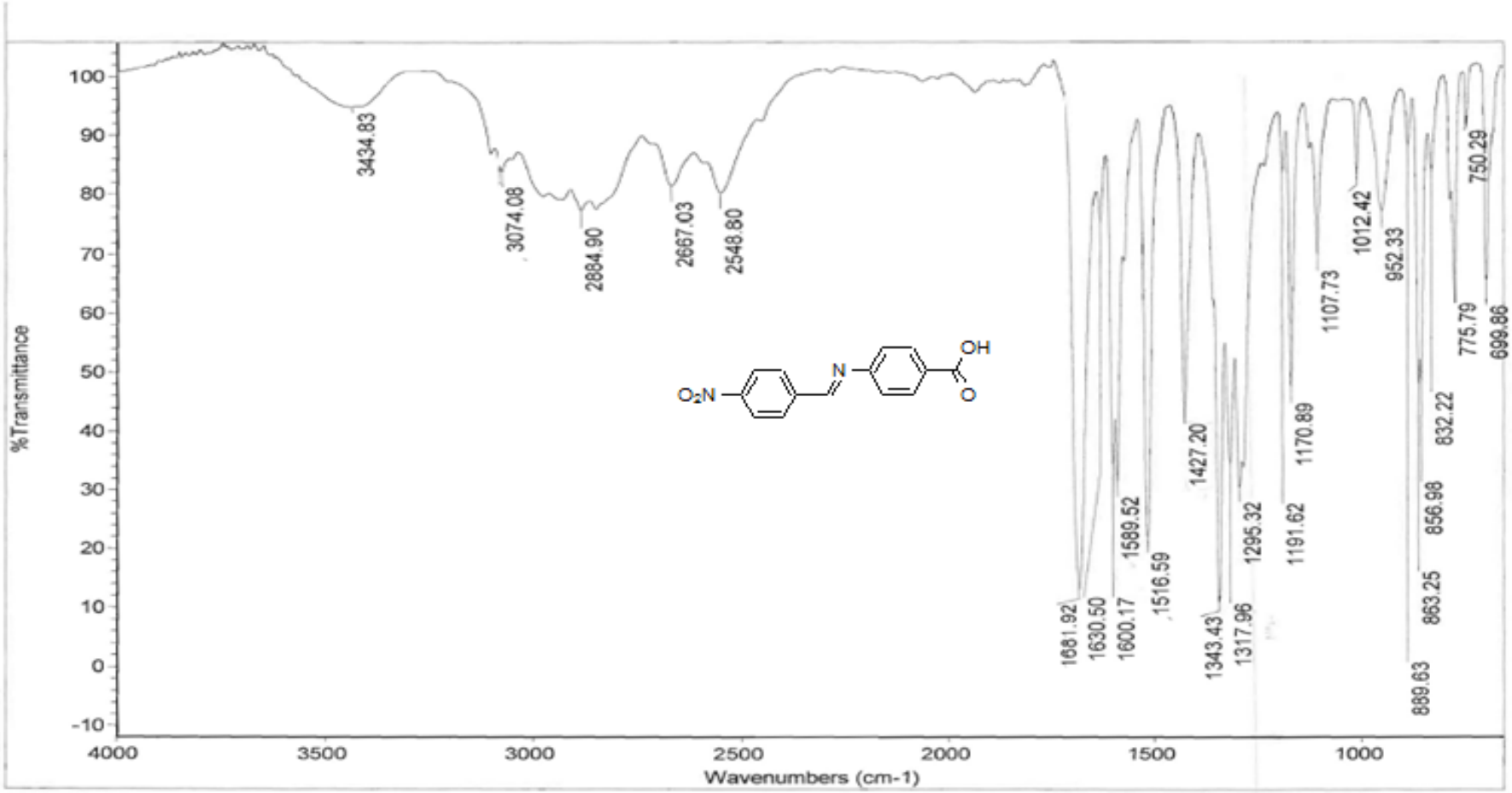


Fig- 44: IR Spectrum of compound SB_2

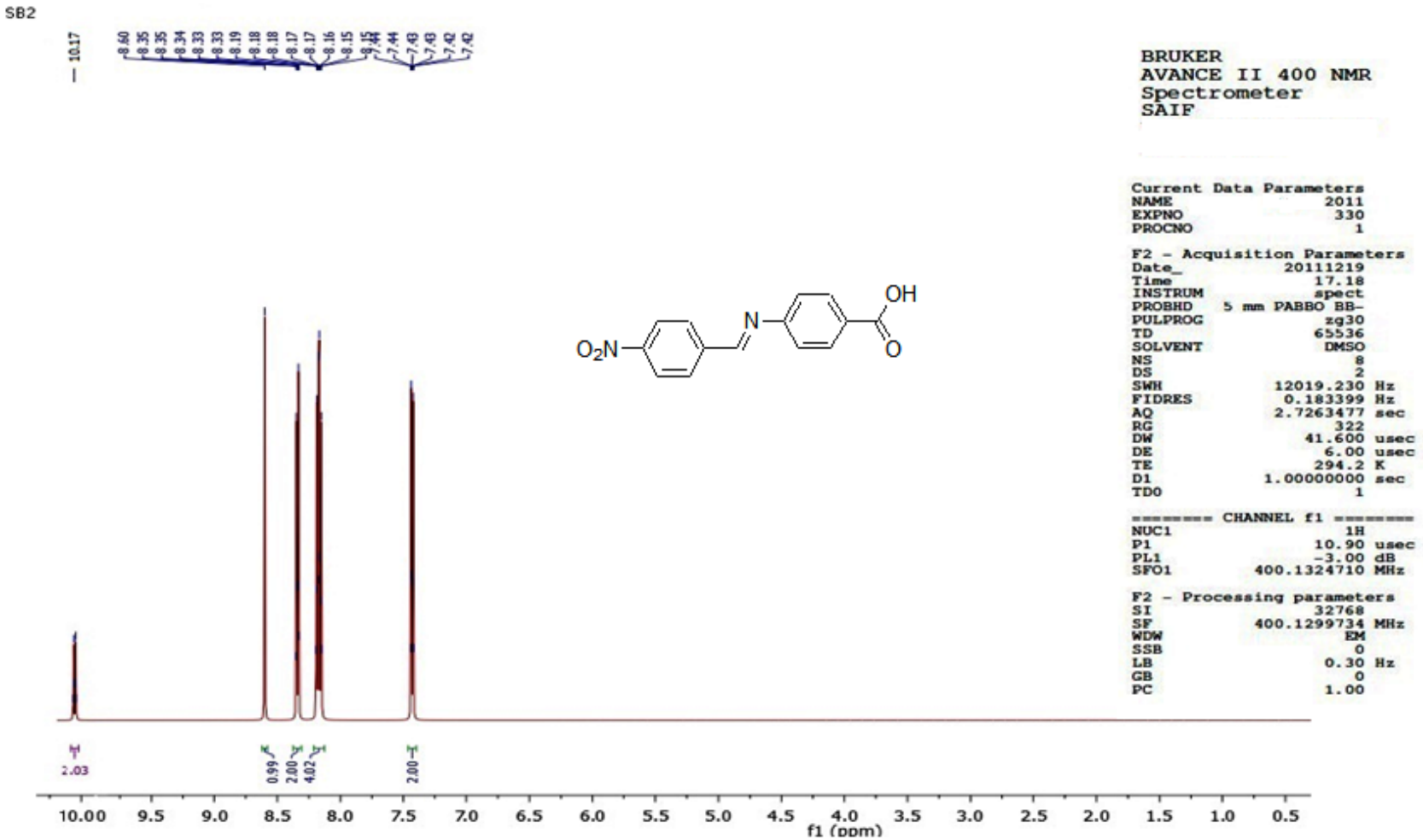


Fig- 45:^{1}H-NMR Spectrum of the compound SB_2

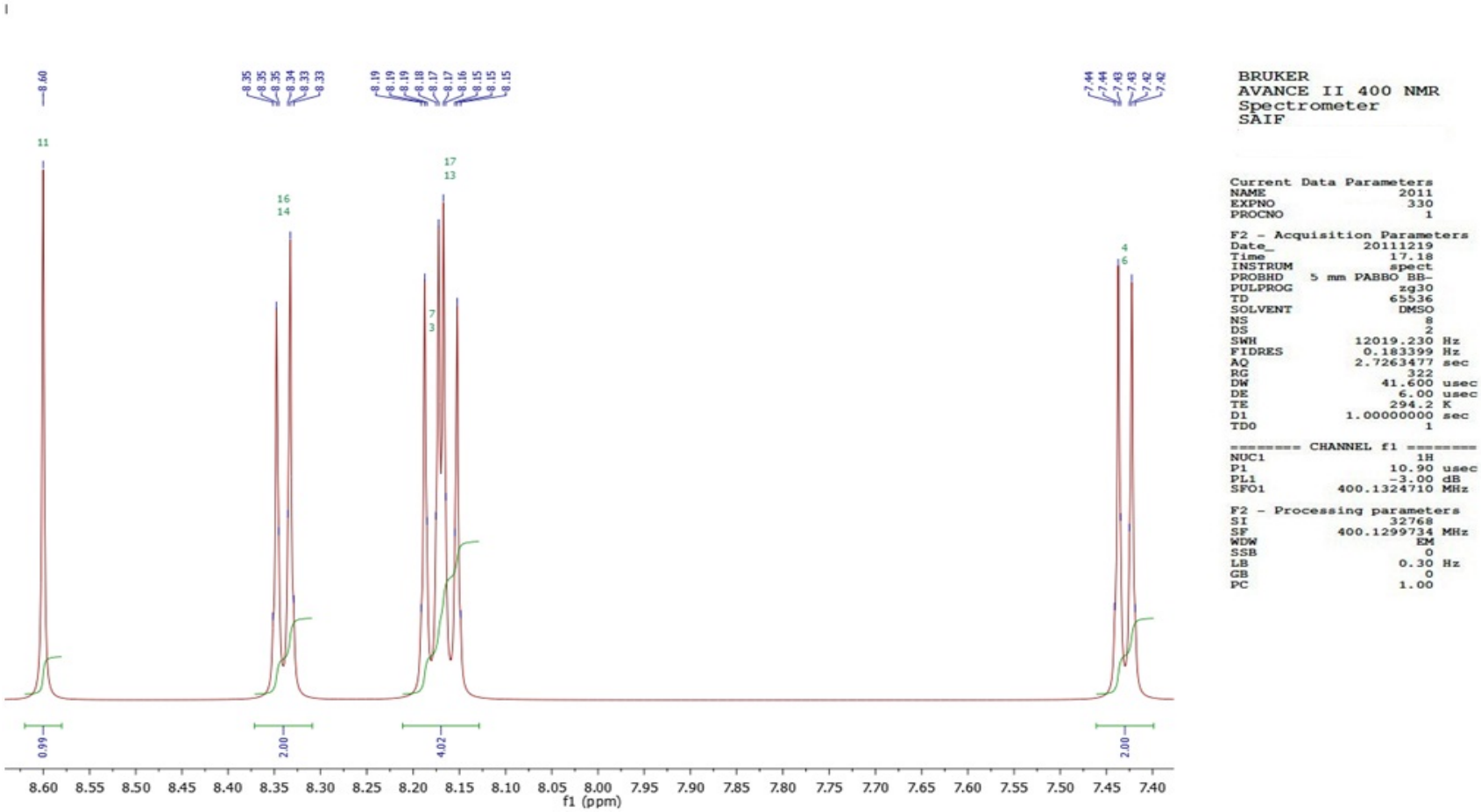


Fig- 46: ^{1}H-NMR Spectrum of the compound of SB_2

6.2.3 Spectral analysis of 4-[4-(hydroxybenzylidene) amino]benzoic acid (SB_3)

UV: (Fig- 47)

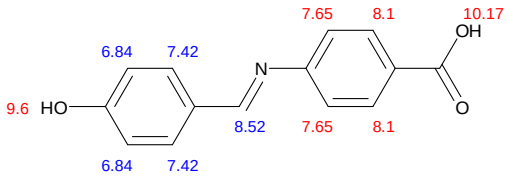
λ_{max} (MeOH) 284.5 (ε_{max} 1.1012)

λ_{max} (MeOH) 220.0 (ε_{max} 0.7090)

IR (KBr): (Fig-48)

Wavelength (cm^{-1})	**Assignment**
3240.51	O-H (stretching)
2885.78	Aromatic C-H (stretching)
1685.99	C=N (stretching)
1575.44	Aromatic C=C (stretching)
1421.83	C-O-H (bending)
1285.44	C-O (stretching)
835.16	Aromatic C-H (bending)

NMR (DMSO-d_6): (Fig-49-50)



(8 aromatic protons, 1 hydroxy proton, 1 proton on Schiff base and 1 proton on carboxylic acid)

δ	**Assignment**
10.65	(1H, s, Ar-COOH)
9.68	(1H, s, Ar-OH)
8.52	(1H, s, CH=N)
8.17-7.65	(4H, m, Ar-H of benzoic acid)
7.42-6.84	(4H, m, Ar-H of hydroxyl phenyl group)

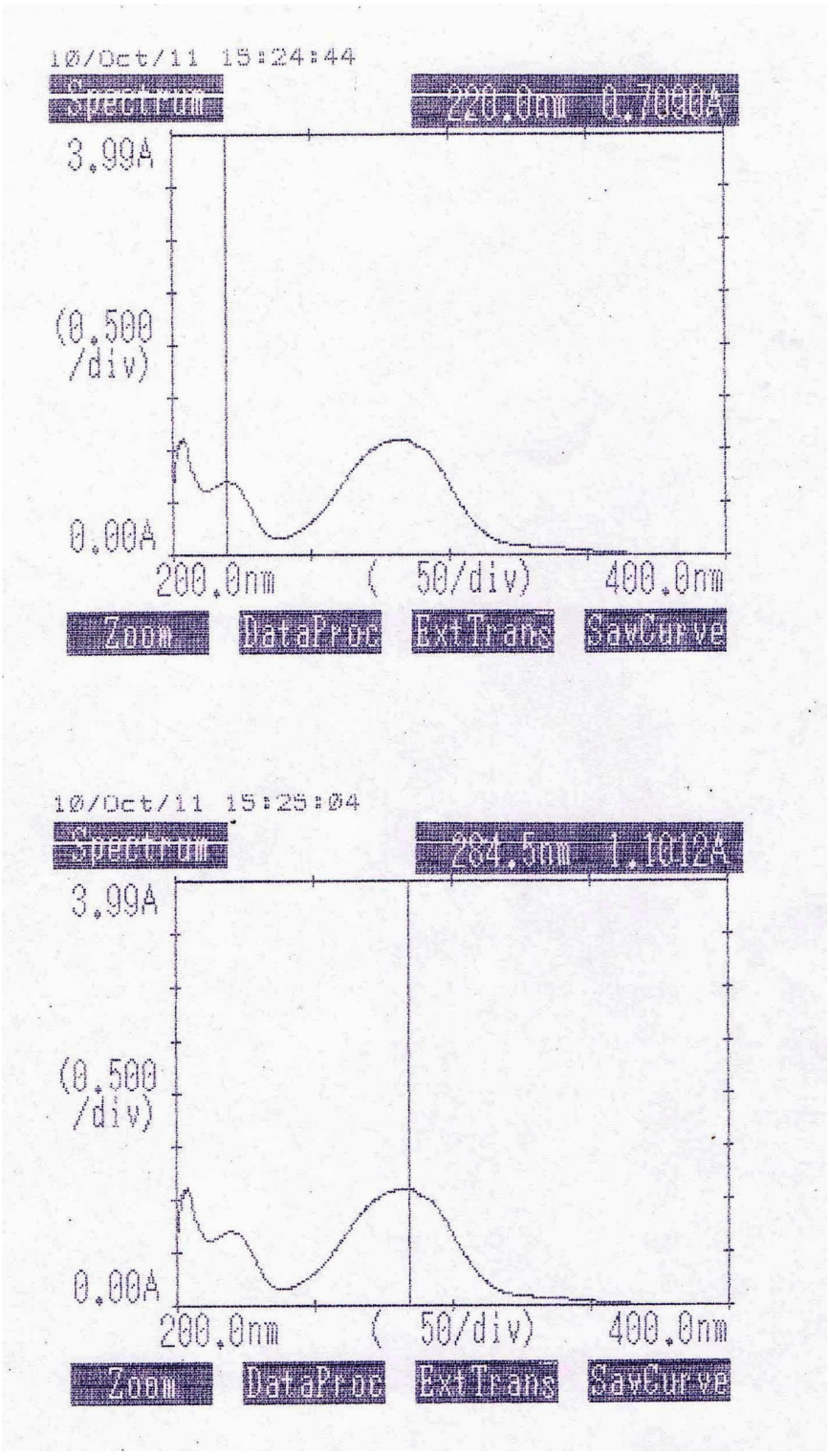


Fig-47 : UV Spectrum of compound SB_3

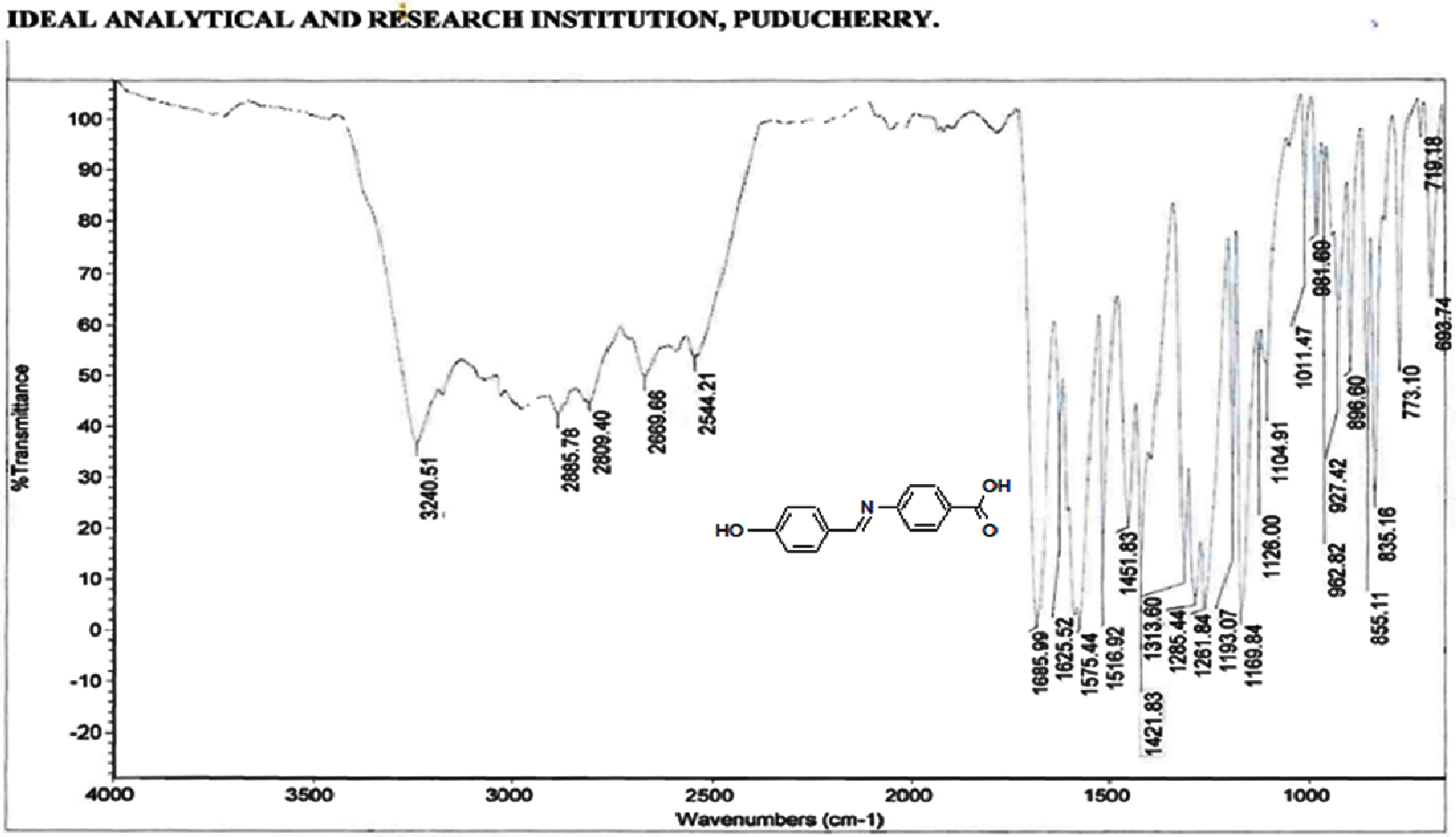


Fig- 48: IR Spectrum of compound SB_3

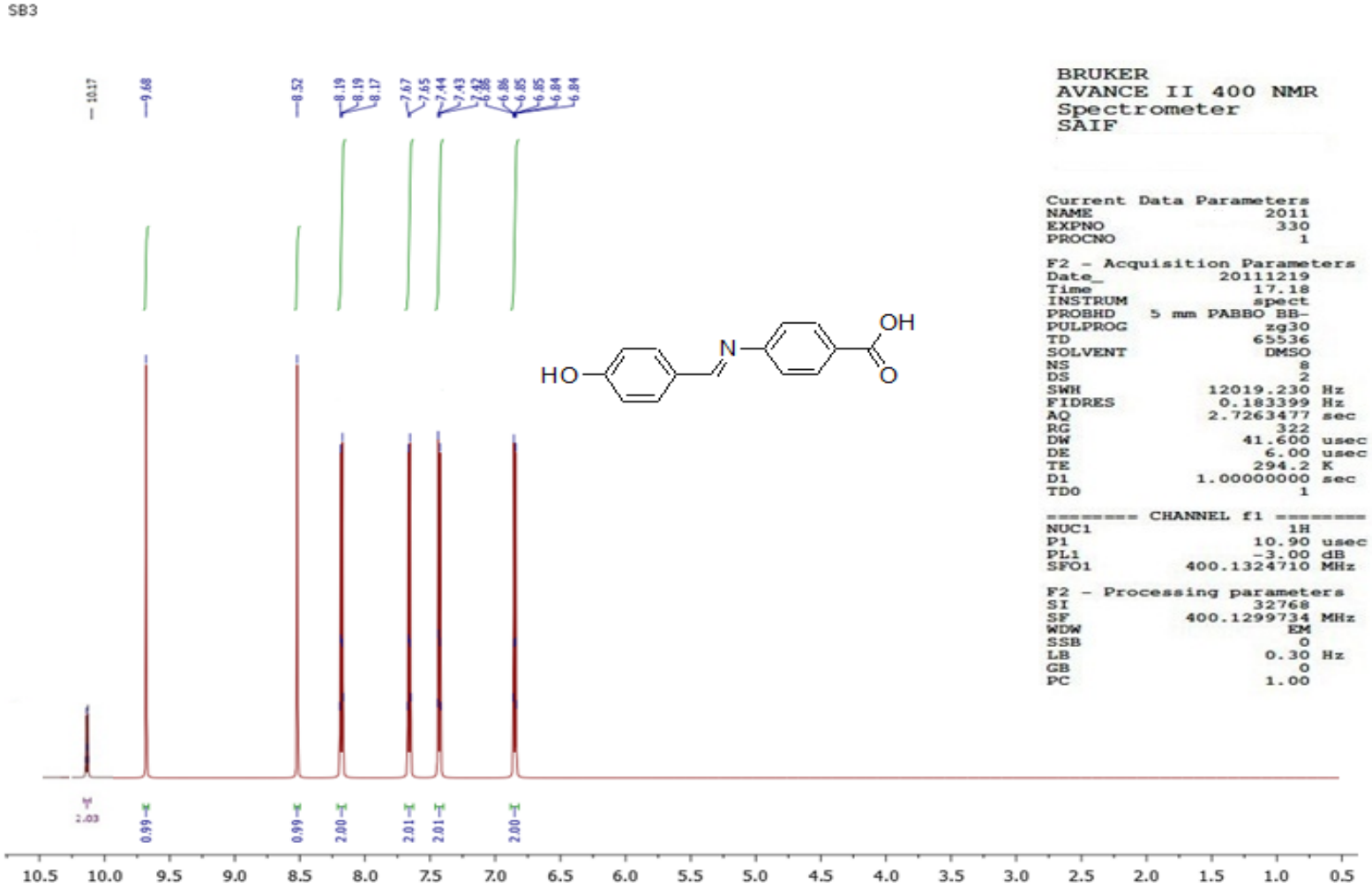


Fig- 49:^{1}H-NMR Spectrum of the compound SB_3

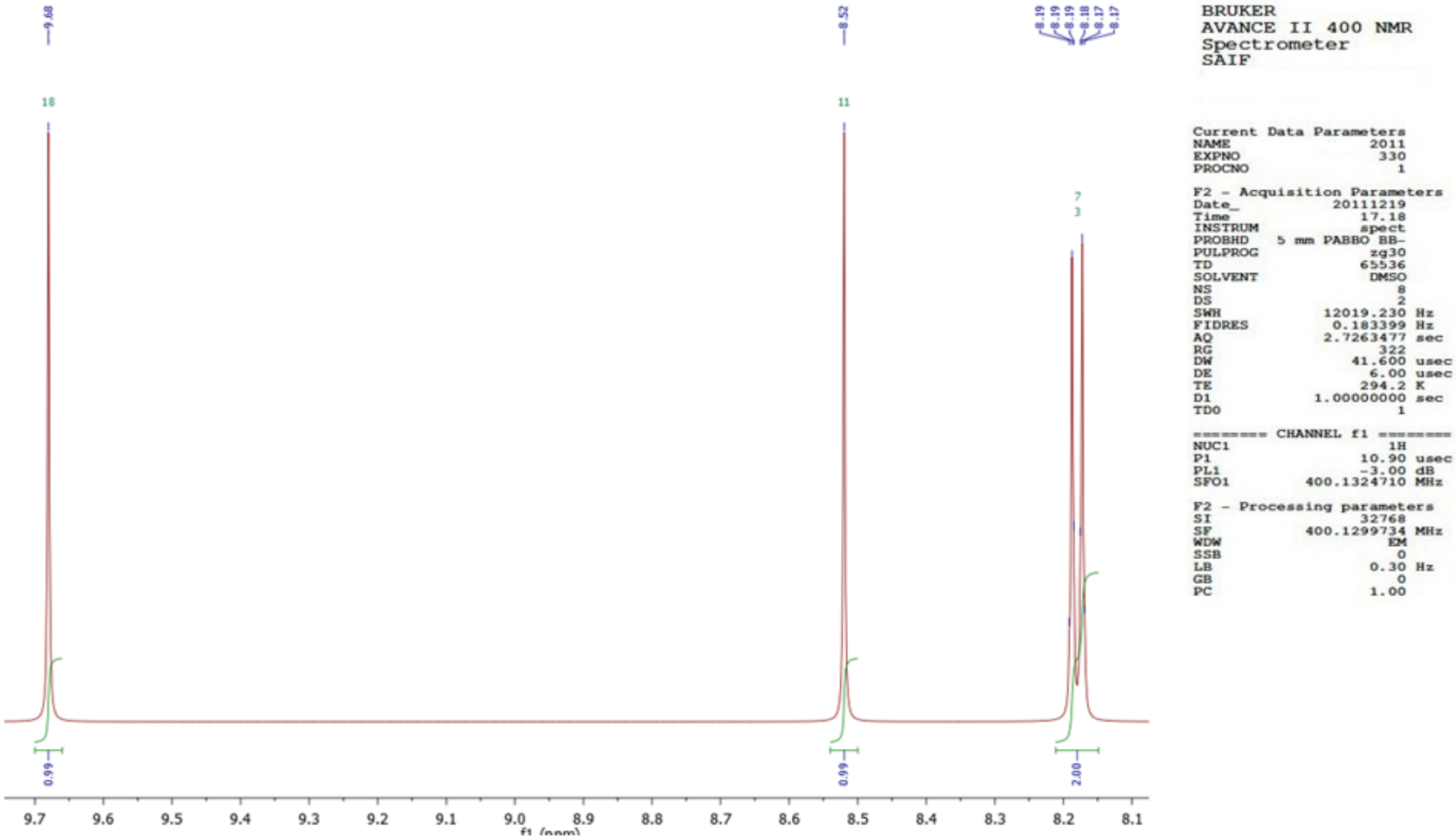


Fig- 50: ^{1}H-NMR Spectrum of the compound SB_3

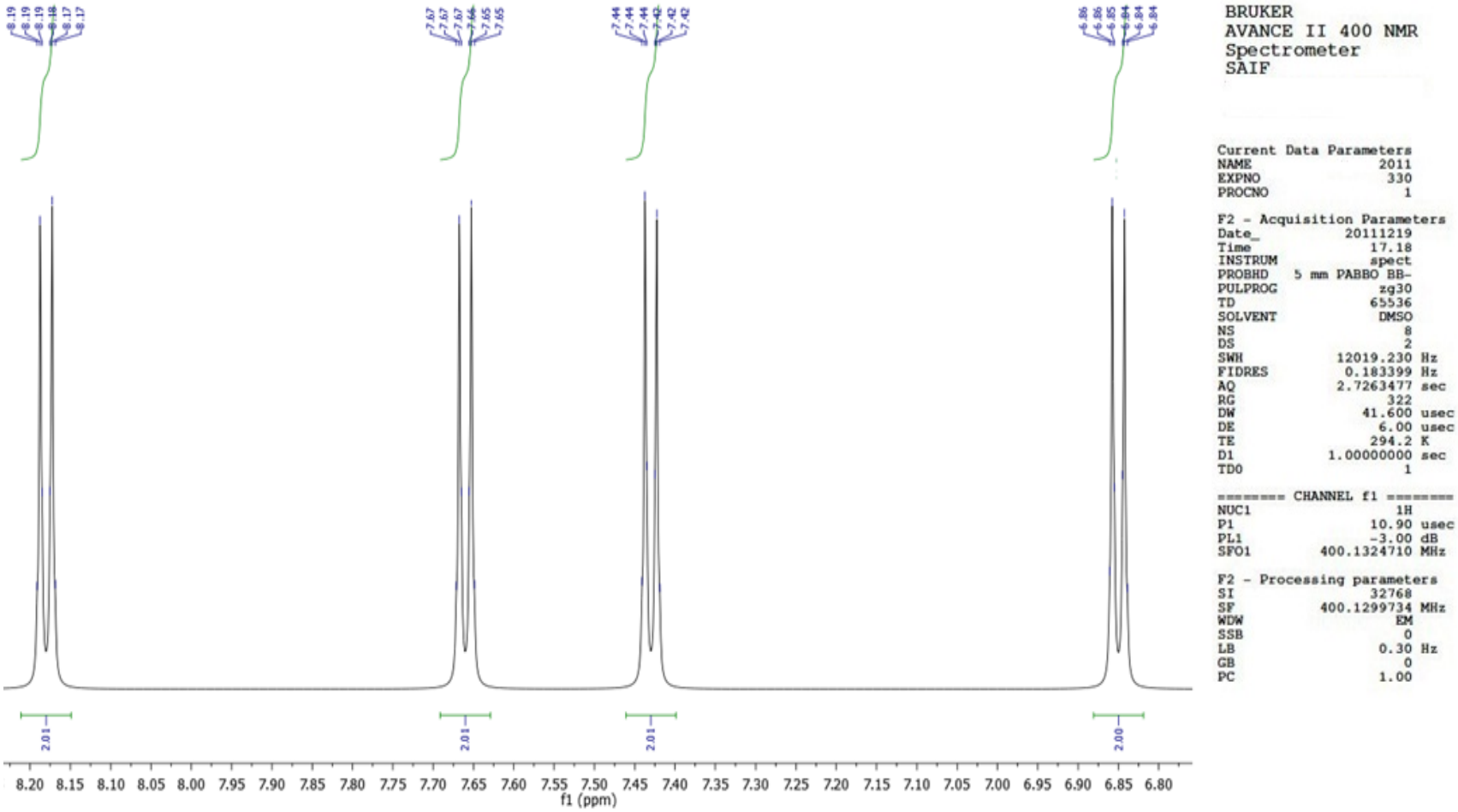


Fig- 51: ^{1}H-NMR Spectrum of the compound SB_3

6.2.4 Spectral analysis of 4-[3-chloro-2-[4-dimethylaminophenyl]-4-oxoazetidin-1-yl]benzoic acid (SS_1)

UV: (Fig- 52)

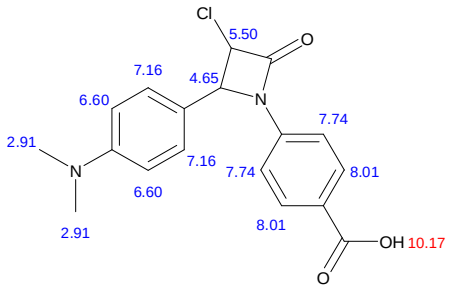
λ_{max} (MeOH) 339.5 (ε_{max} 1.4282)

λ_{max} (MeOH) 285.5 (ε_{max} 1.0659)

IR (KBr): (Fig- 53)

Wavelength (cm^{-1})	**Assignment**
2916.76	Aromatic C-H (stretching)
1659.04	C=O azetidinone(stretching)
1600.46	Aromatic C=C (stretching)
1436.72	C-O-H (bending)
1366.95	C-N (stretching)
1436.72	N-CH_3 (stretching)
1285.86	C-O (stretching)
856.09	Aromatic C-H (bending)
773.83	C-Cl (stretching)

NMR (DMSO-d_6): (Fig-54-56)

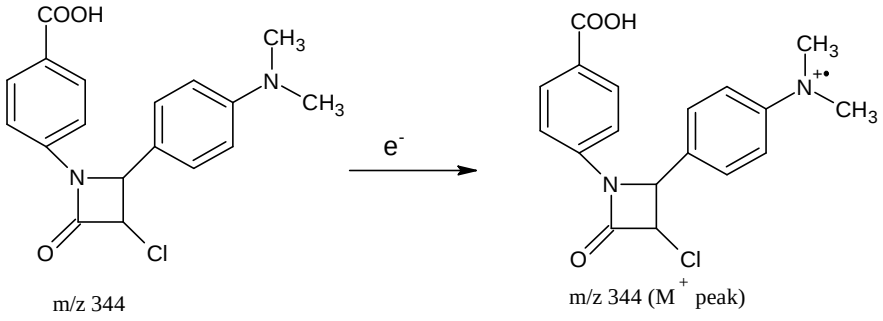


(8 aromatic protons, 6 methyl protons, 2 cyclic protons and 1 proton on carboxylic acid)

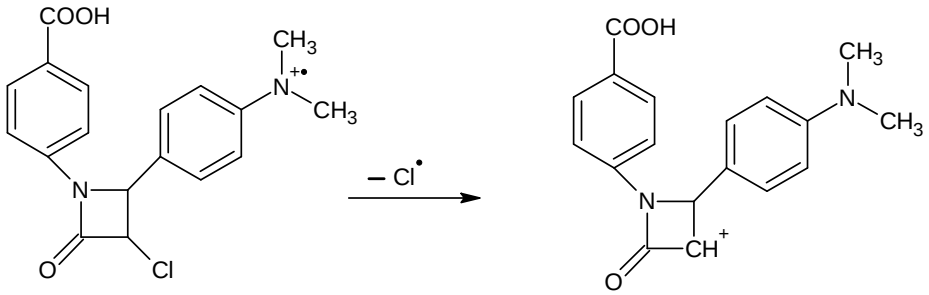
δ	Assignment
10.17	(1H, s, Ar-COOH)
8.01-7.74	(4H, m, Ar-H of benzoic acid)
7.16-6.60	(4H, m, Ar-H of dimethyl amino phenyl)
5.50	(1H. d, CH-Cl)
4.65	(1H. d, CH)
2.91	(6H, s, $N(CH_3)_2$)

MASS: (Fig-57)

The structure of the compound was further confirmed by its fragmentation peaks which are as follows:



m/z 344 m/z 344 (M^+ peak)



m/z 344 (M^+ peak) m/z 309

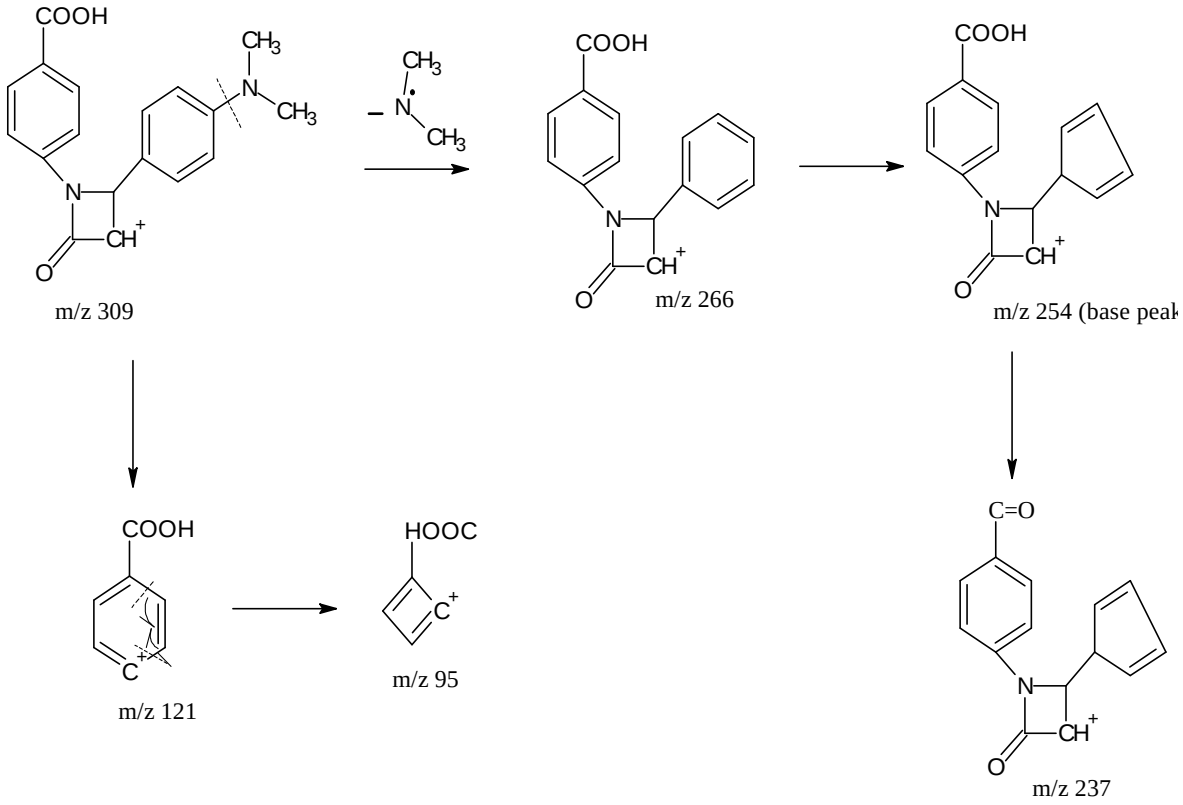


Fig- 58: Fragmentation of 4-[3-chloro-2-[4-dimethylaminophenyl]-4-oxoazetidin-1-yl] benzoic acid

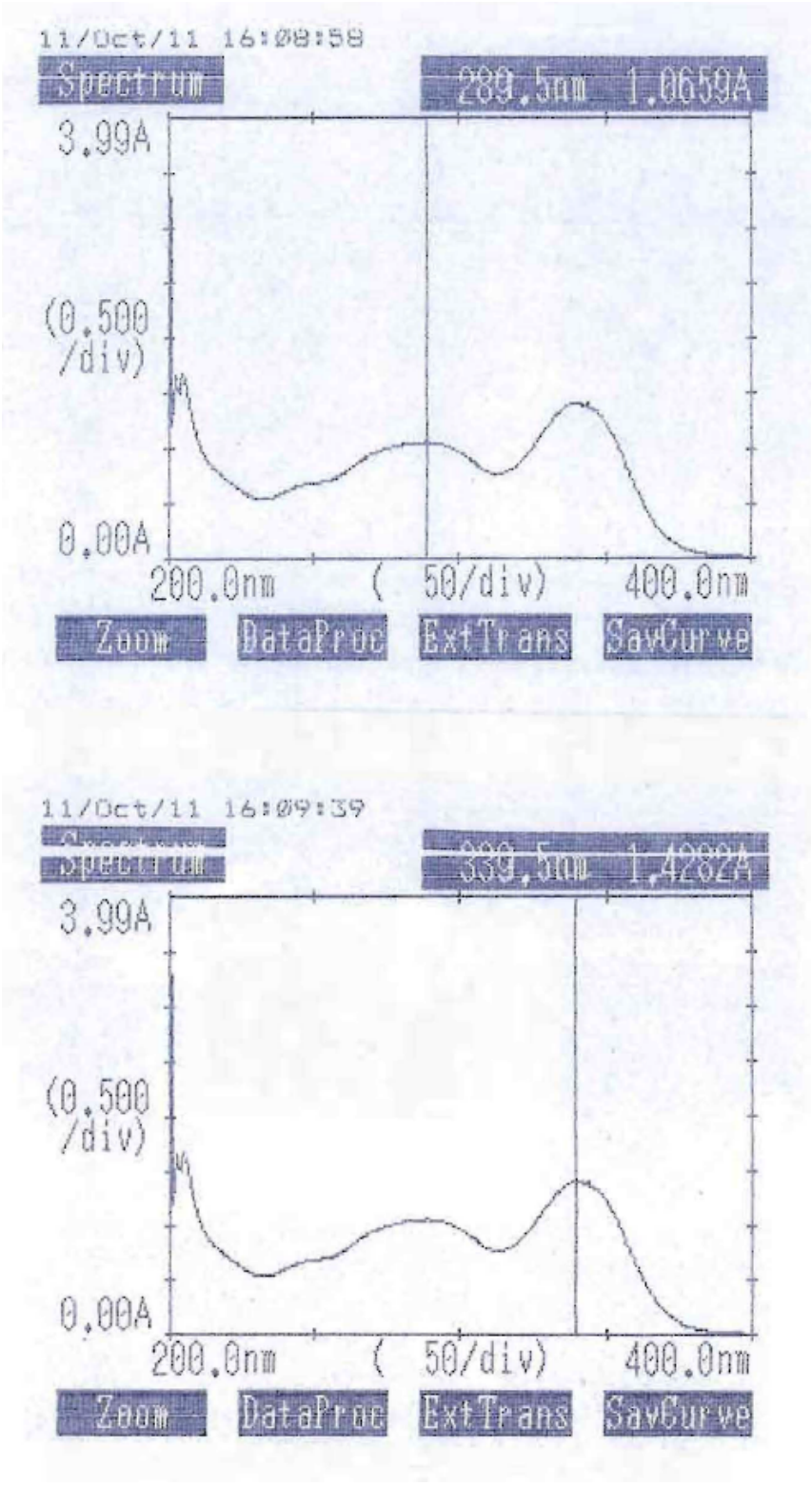


Fig-52: UV Spectrum of compound SS_1

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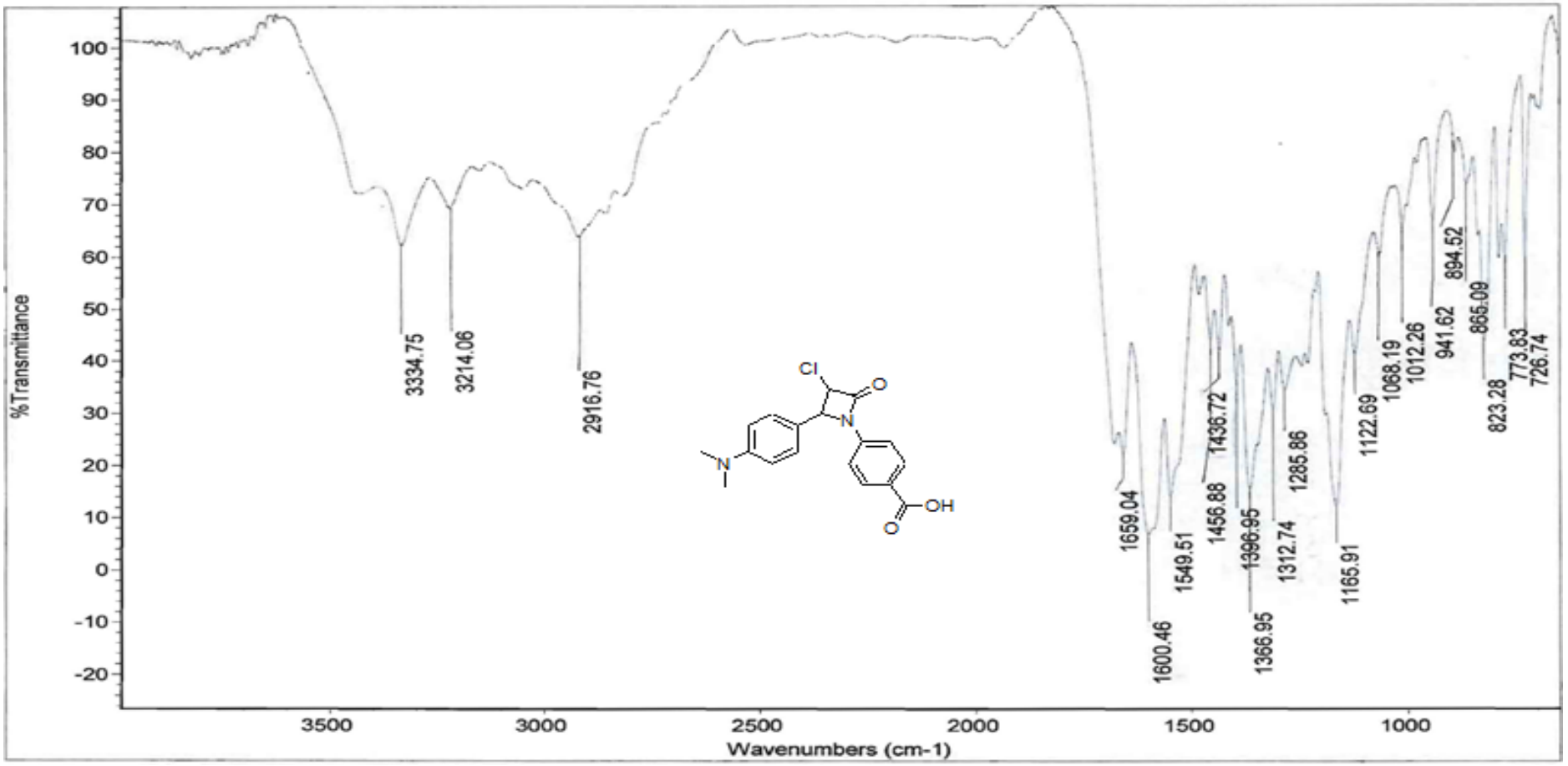


Fig-53: IR Spectrum of compound SS_1

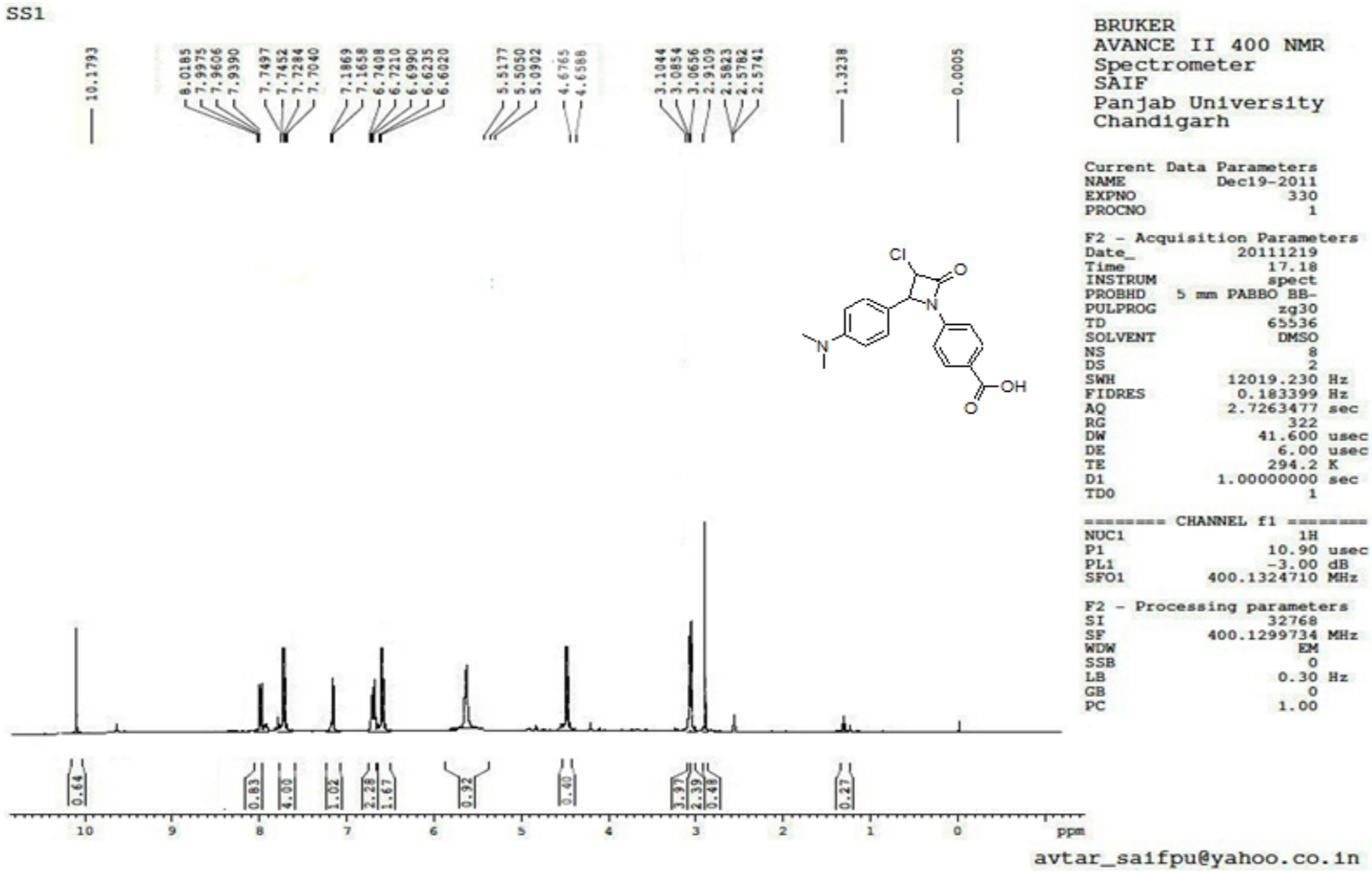


Fig-54: ^{1}H-NMR Spectrum of the compound SS_1

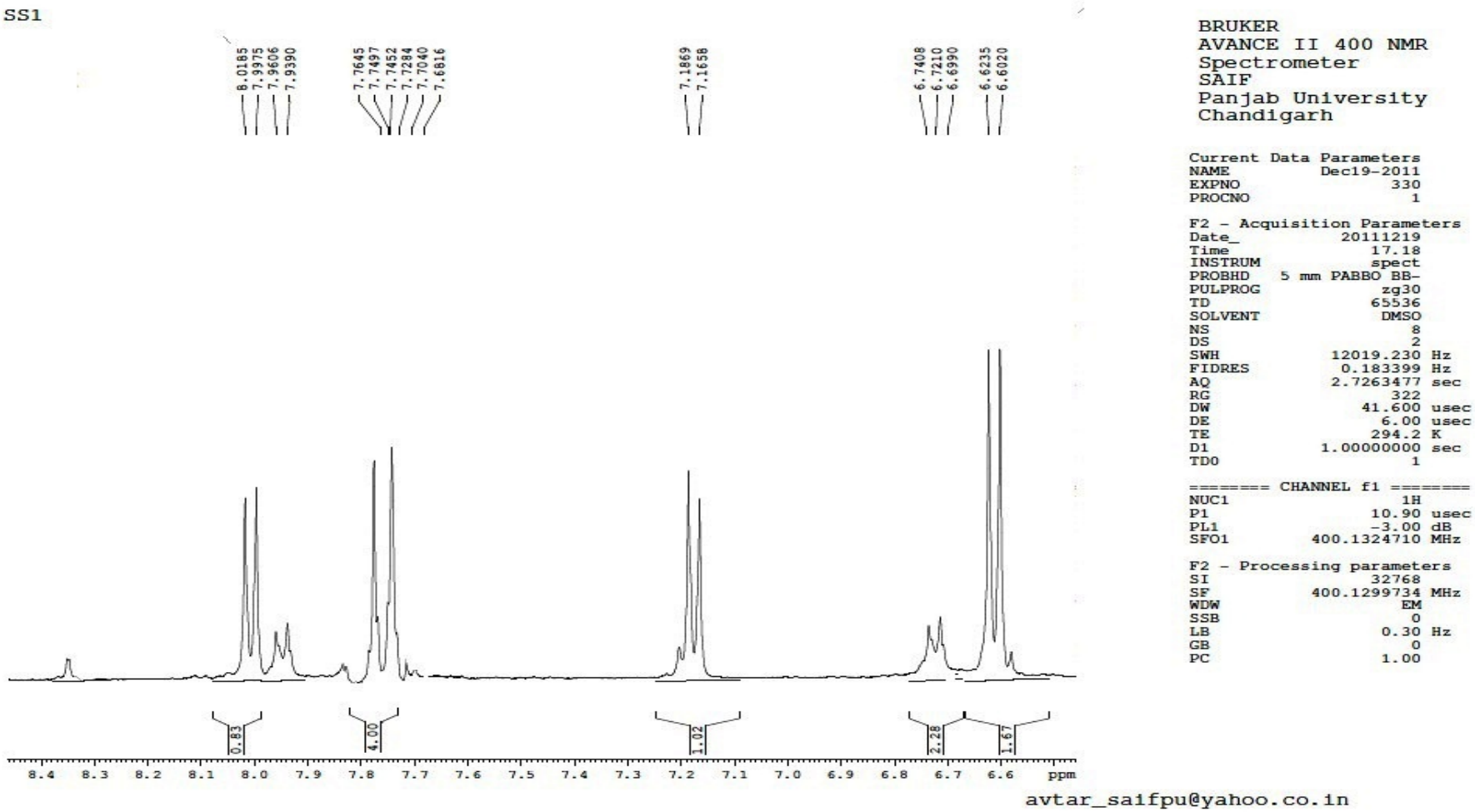


Fig-55: ^{1}H-NMR Spectrum of the compound SS_1 (Zoom View 1)

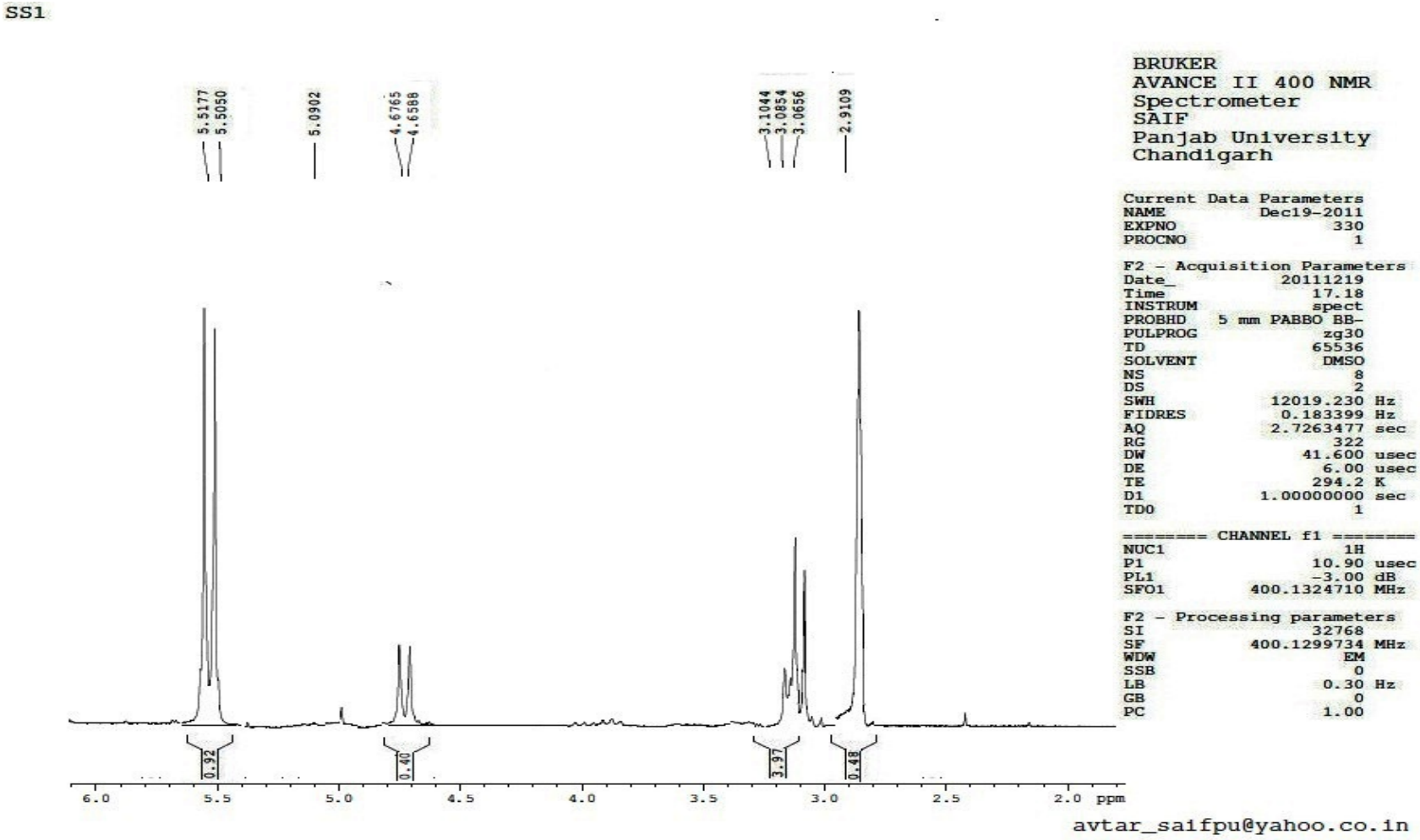


Fig-56: ^{1}H-NMR Spectrum of the compound SS_1 (Zoom View 2)

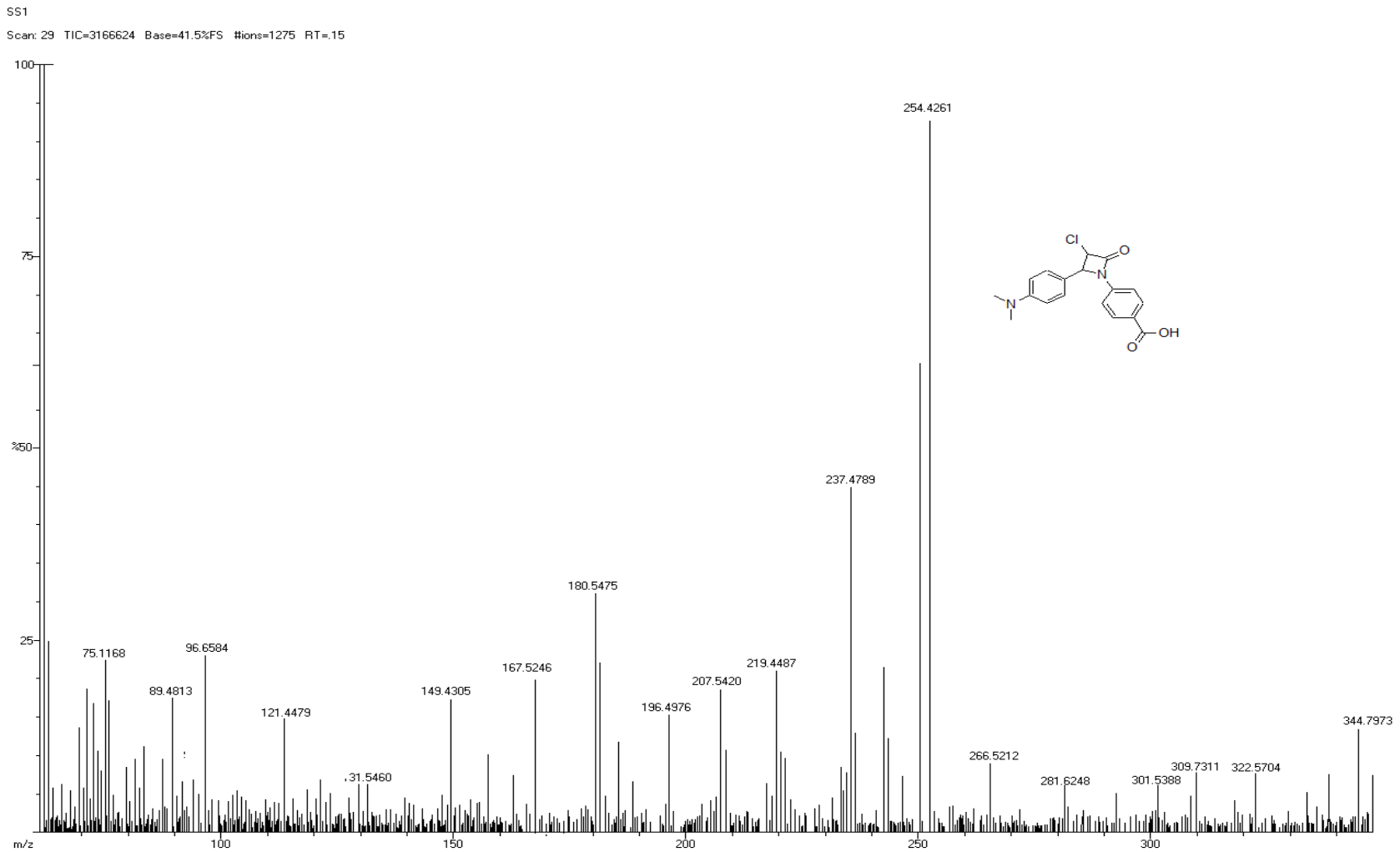


Fig-57: Mass Spectrum of the compound SS_1

6.2.2 Spectral analysis of 4-[3-chloro-2-(4-nitrophenyl)-4-oxoazetidin-1-yl]-benzoic acid (SS_2)

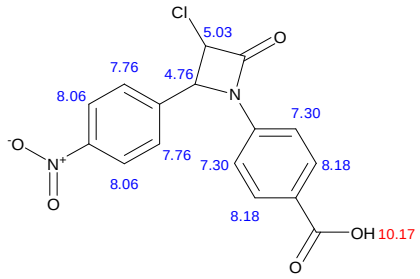
UV: (Fig-59)

λ_{max} (MeOH) 175.5 (ε_{max} 1.5068)

IR (KBr): (Fig-60)

Wavelength (cm^{-1})	Assignment
3074.16	Aromatic C-H (stretching)
1682.41	C=O azetidinone(stretching)
1600.74	Aromatic C=C (stretching)
1517.57	Aromatic NO_2 asymmetric (stretching)
1426.40	C-O-H (bending)
1344.94	Aromatic NO_2 symmetric (stretching)
1317.05	C-N (stretching)
1294.66	C-O (stretching)
857.10	Aromatic C-H (bending)
775.27	C-Cl (stretching)

NMR (DMSO-d_6): (Fig-61-63)

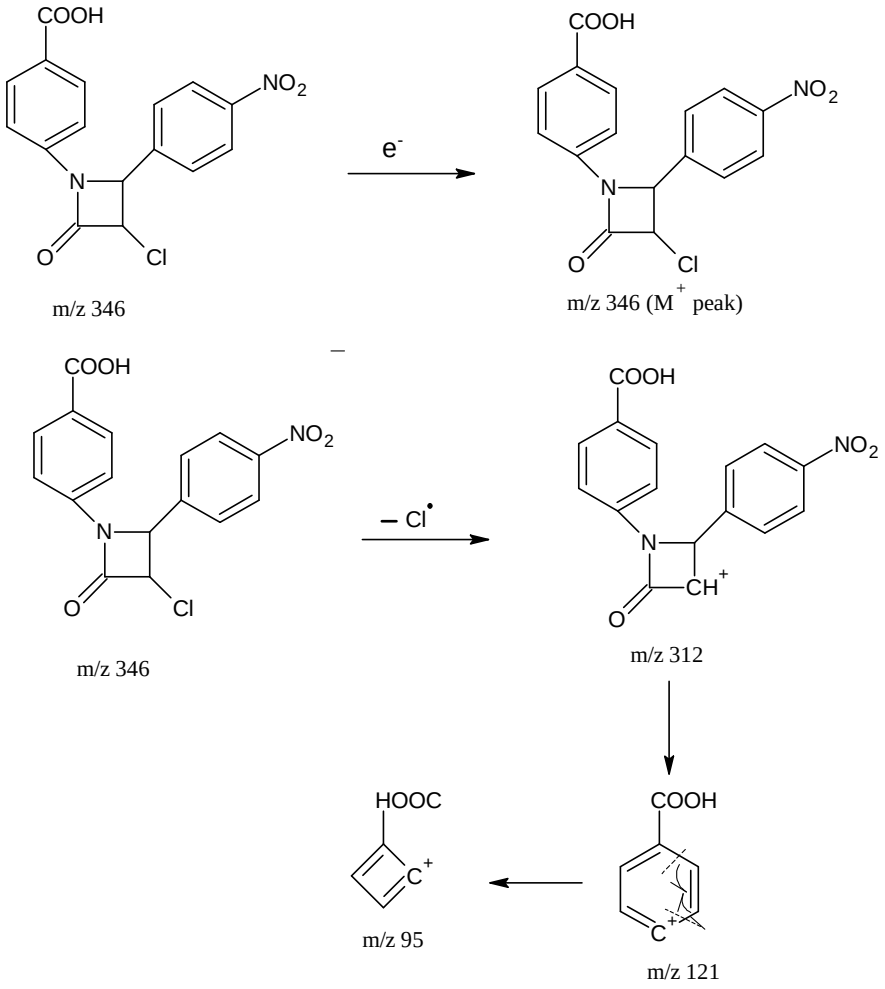


(8 aromatic protons, 2 cyclic protons and 1 proton on carboxylic acid)

δ	Assignment
10.17	(1H, s, Ar-COOH)
8.06-7.30	(8H, m, Ar-H)
5.03	(1H. d, CH-Cl)
4.76	(1H. d, CH)

MASS: (Fig-64)

The structure of the compound was further confirmed by its fragmentation peaks which are as follows:



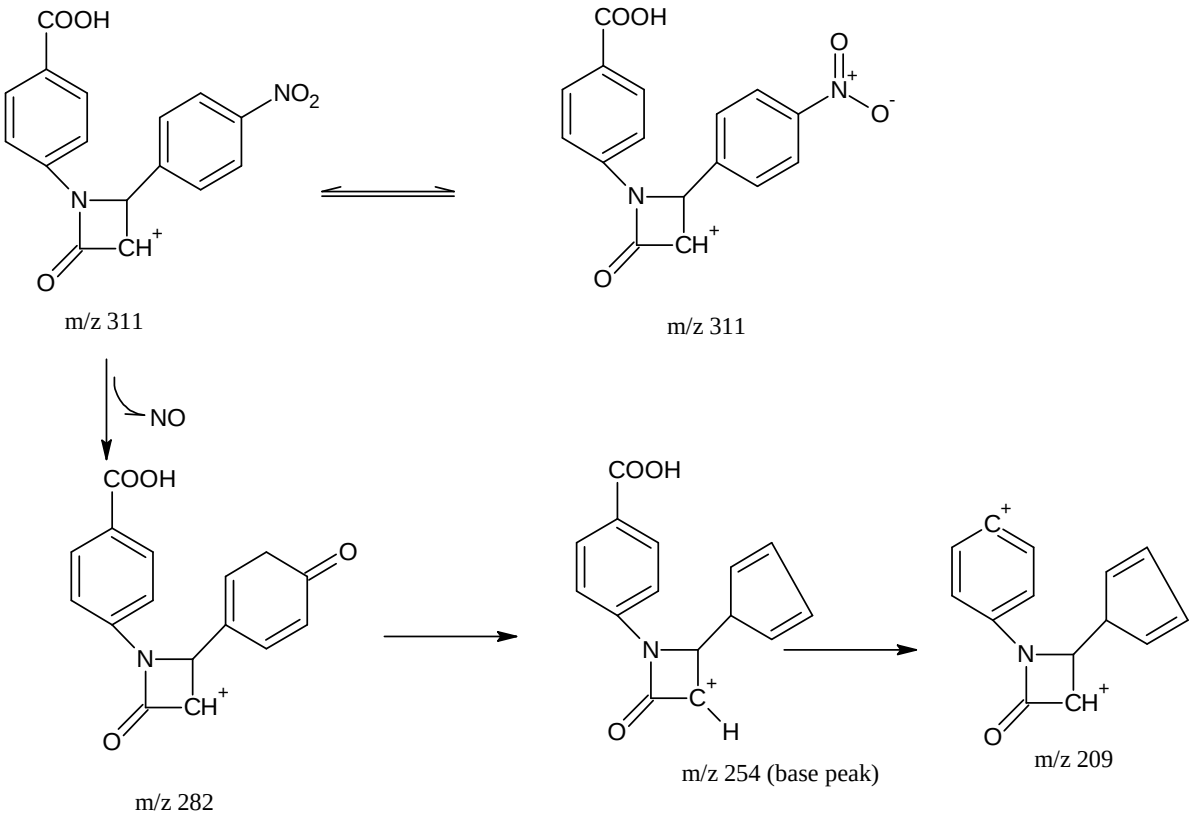


Fig-65: Fragmentation of 4-[3-chloro-2-(4-nitrophenyl)-4-oxoazetidin-1-yl]benzoic acid

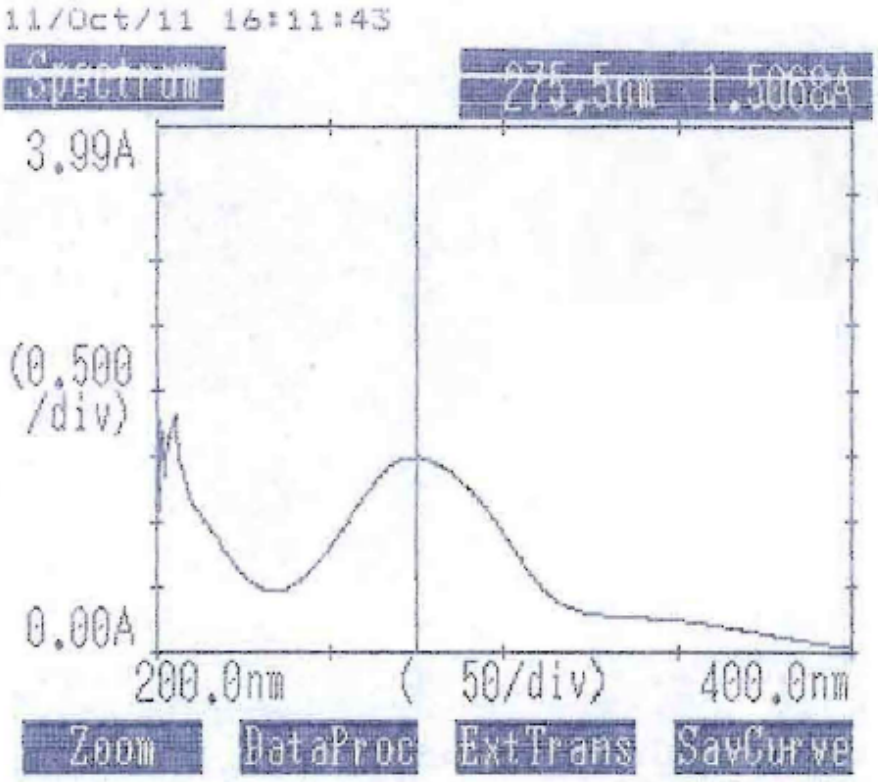


Fig-59: UV Spectrum of compound SS_2

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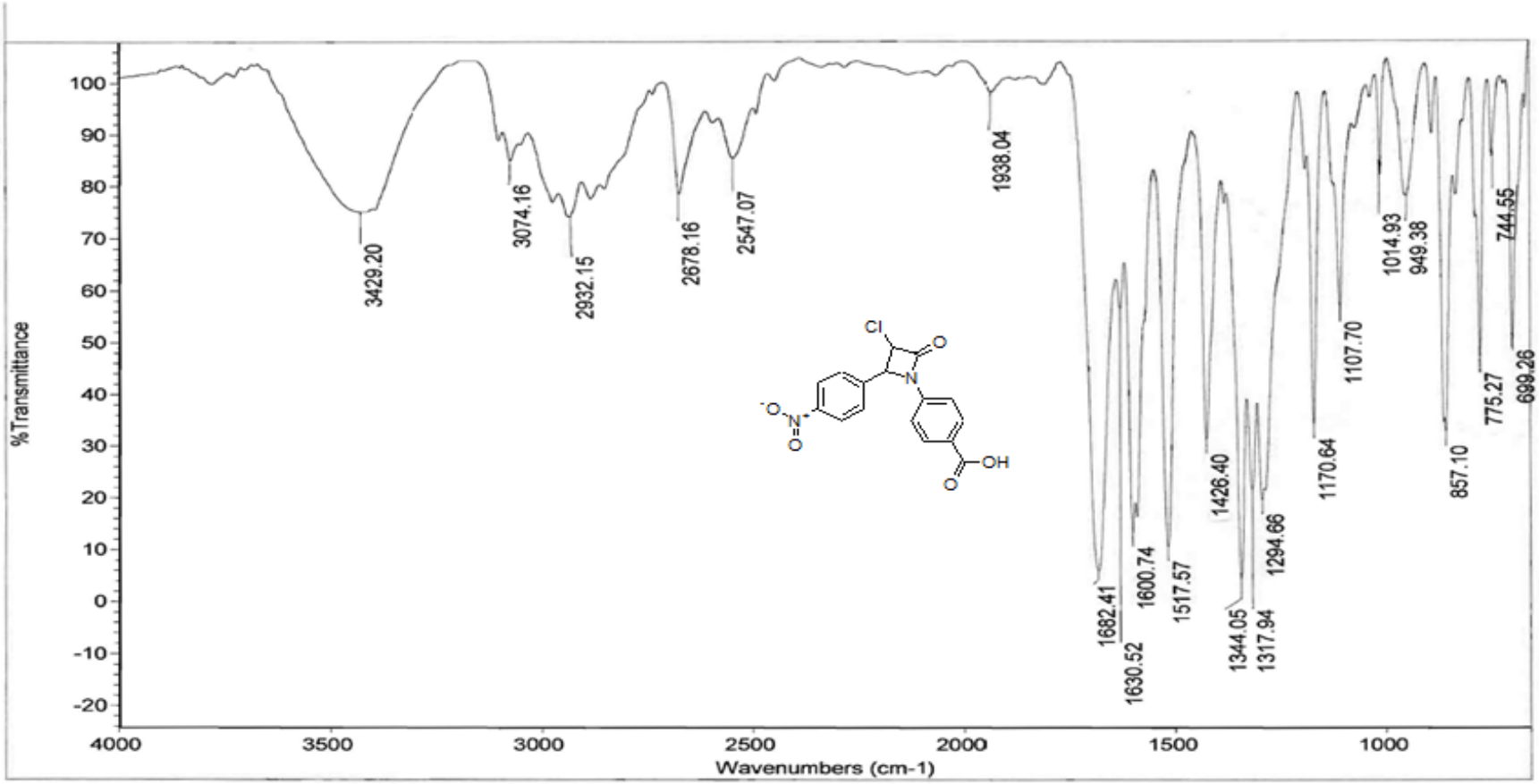


Fig-60: IR Spectrum of compound SS_2

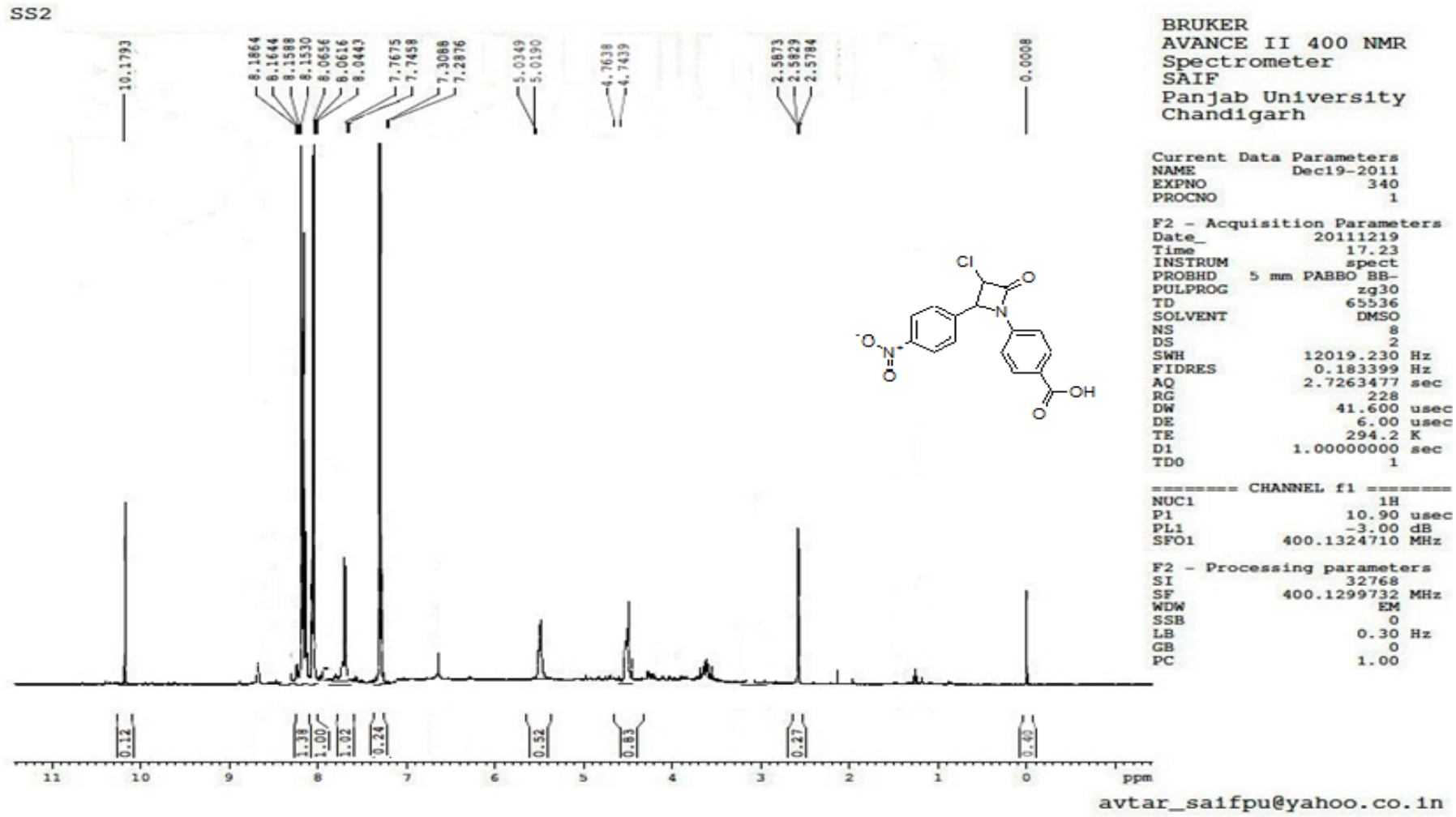


Fig-61: ^{1}H-NMR Spectrum of the compound SS_2

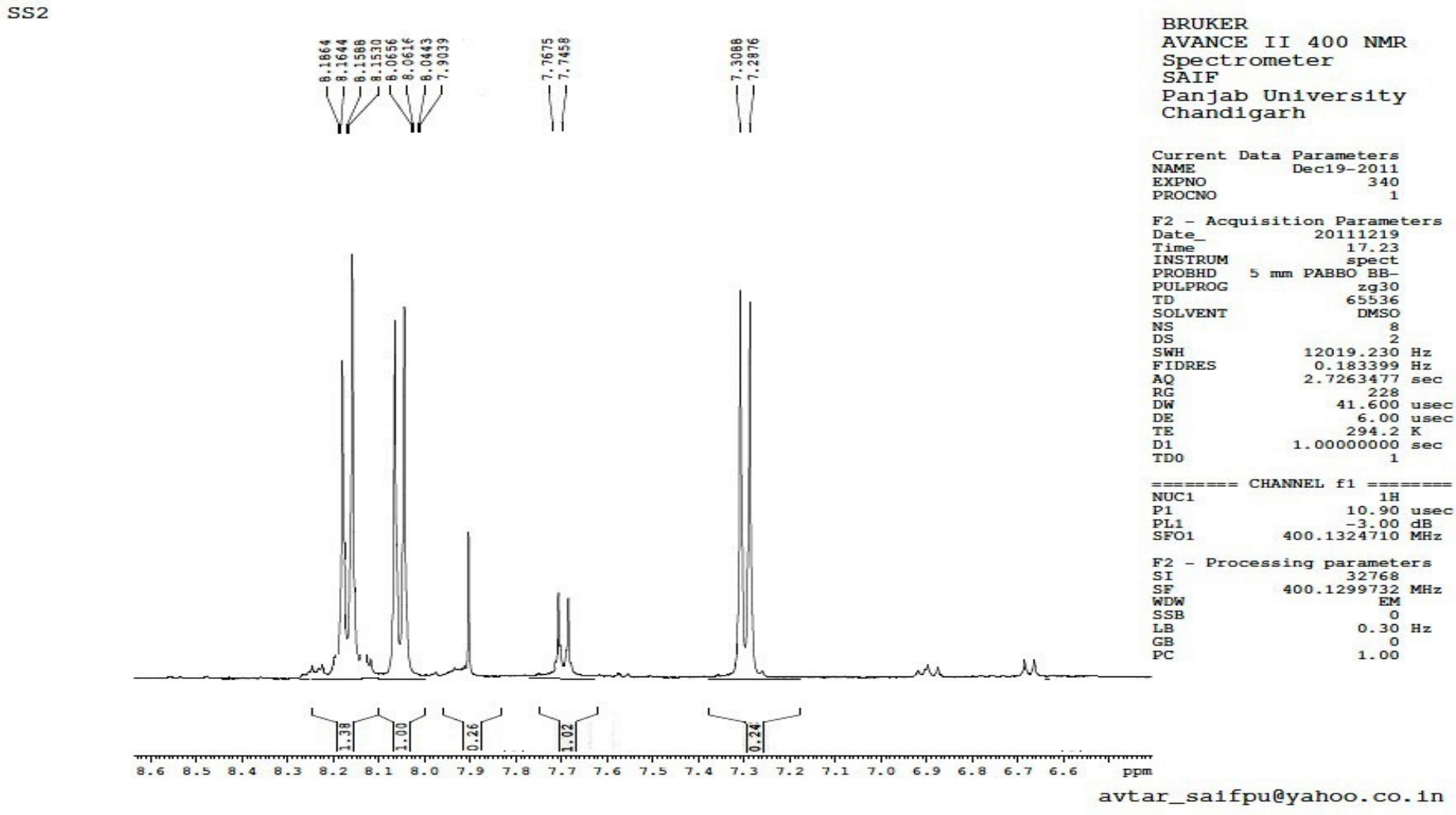


Fig-62: ^{1}H-NMR Spectrum of the compound SS_2(Zoom View 1)

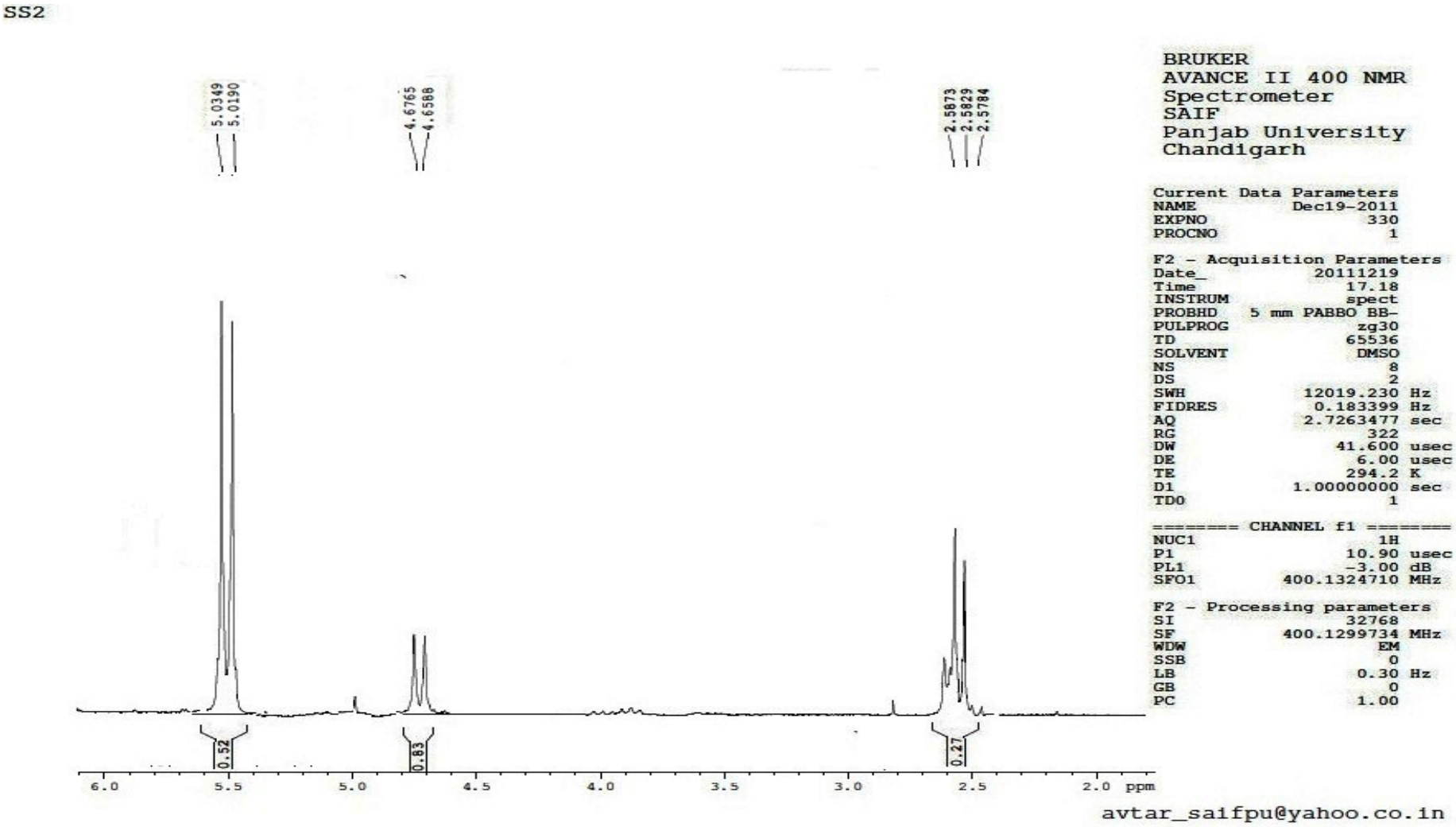


Fig-63: ^{1}H-NMR Spectrum of the compound SS_2(Zoom View 3)

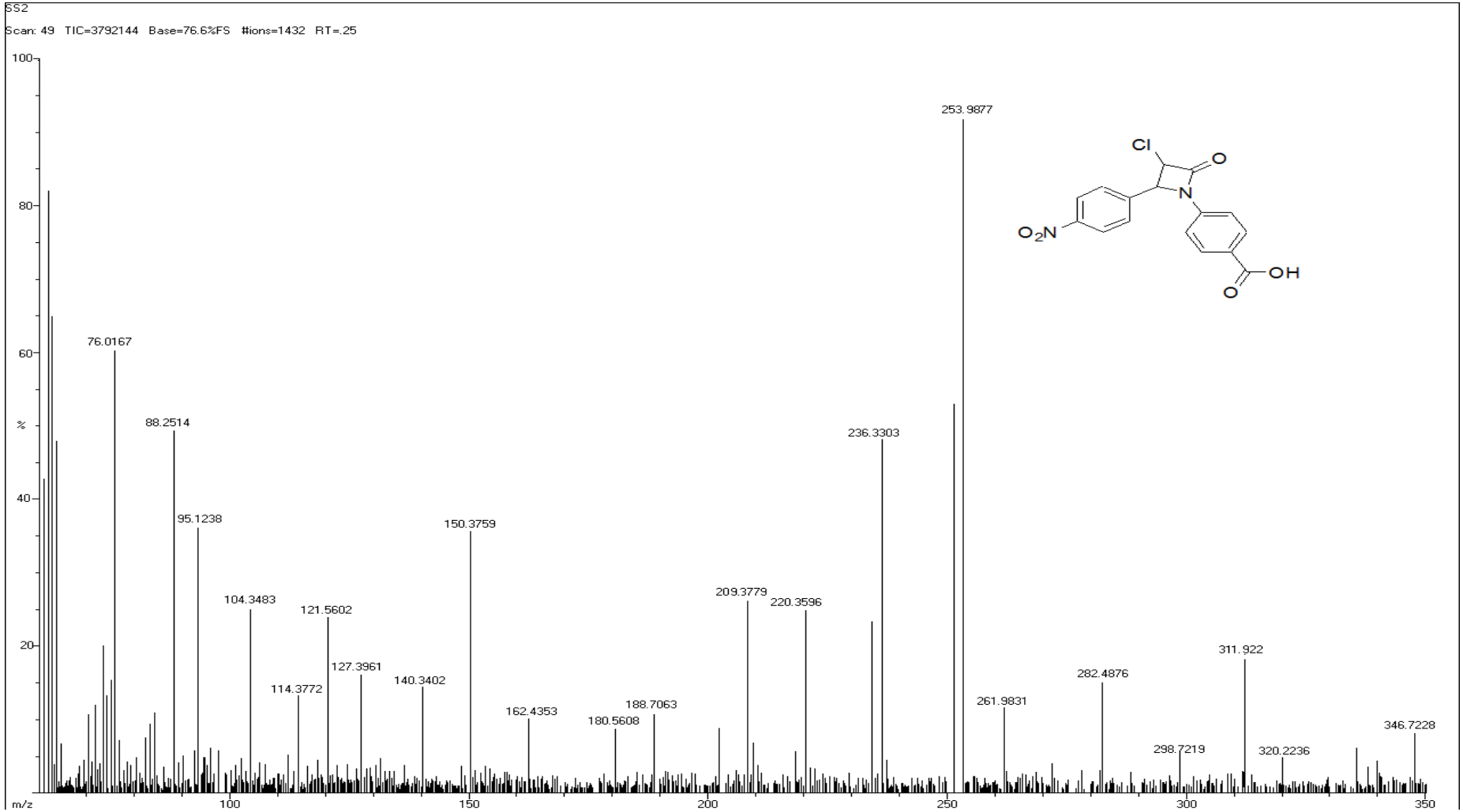


Fig-64: Mass Spectrum of the compound SS_2

6.2.3. Spectral analysis of 4-[3-chloro-2-(4-hydroxyphenyl)-4-oxoazetidin-1-yl]-benzoic acid (SS_3)

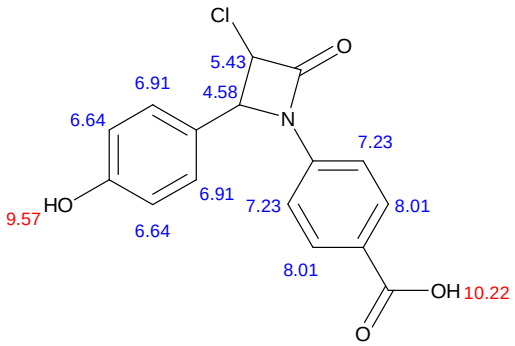
UV: (Fig-66)

λ_{max} (MeOH) 282.0 (ε_{max} 2.6021)

IR (KBr): (Fig-67)

Wavelength (cm^{-1})	**Assignment**
3247.31	OH (stretching)
2966.56	Aromatic C-H (stretching)
1685.31	C=O azetidinone(stretching)
1593.82	Aromatic C=C (stretching)
1421.60	C-O-H (bending)
1313.51	C-N (stretching)
1285.27	C-O (stretching)
857.10	Aromatic C-H (bending)
775.27	C-Cl (stretching)

NMR (DMSO-d_6): (Fig-68 & 69)

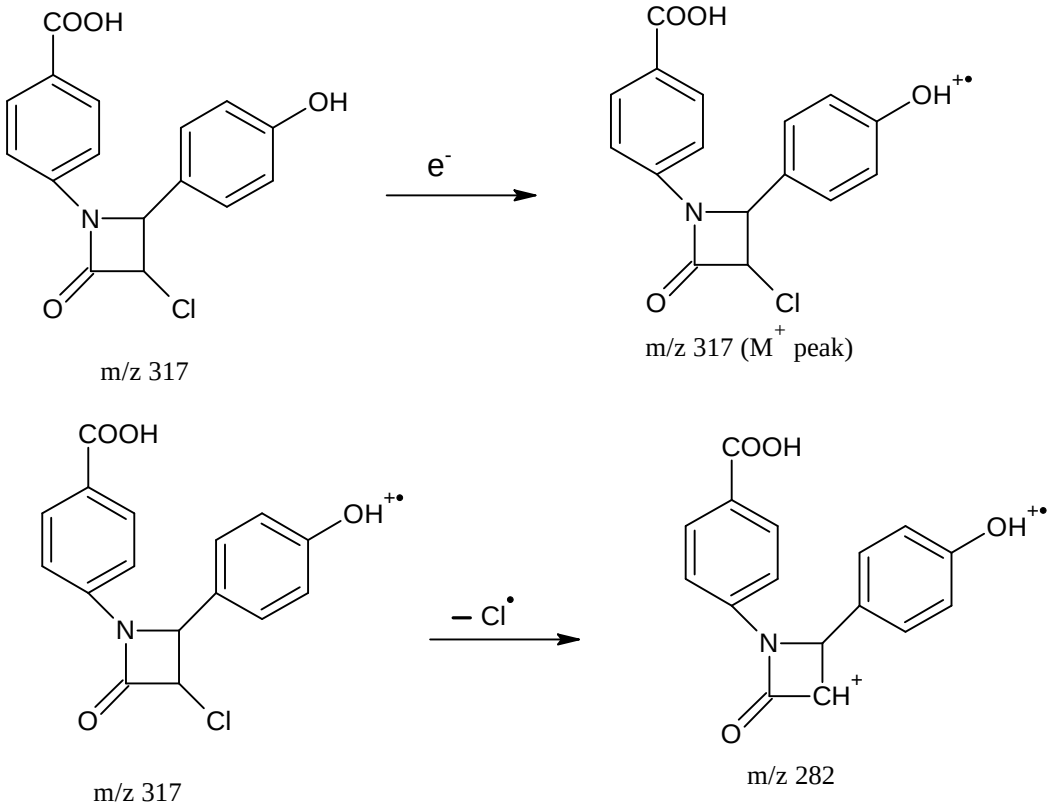


(8 aromatic protons, 2 cyclic protons, 1 hydroxy proton and 1 proton on carboxylic acid)

δ	Assignment
10.22	(1H, s, Ar-COOH)
9.57	(1H, s, Ar-OH)
8.01-7.23	(4H, m, Ar-H of benzoic acid)
6.91-6.64	(4H, m, Ar-H of hydroxyl group)
5.43	(1H. d, CH-Cl)
4.58	(1H. d, CH)

MASS: (Fig-70)

The structure of the compound was further confirmed by its fragmentation peaks which are as follows:



m/z 317

m/z 317 (M^+ peak)

m/z 317

m/z 282

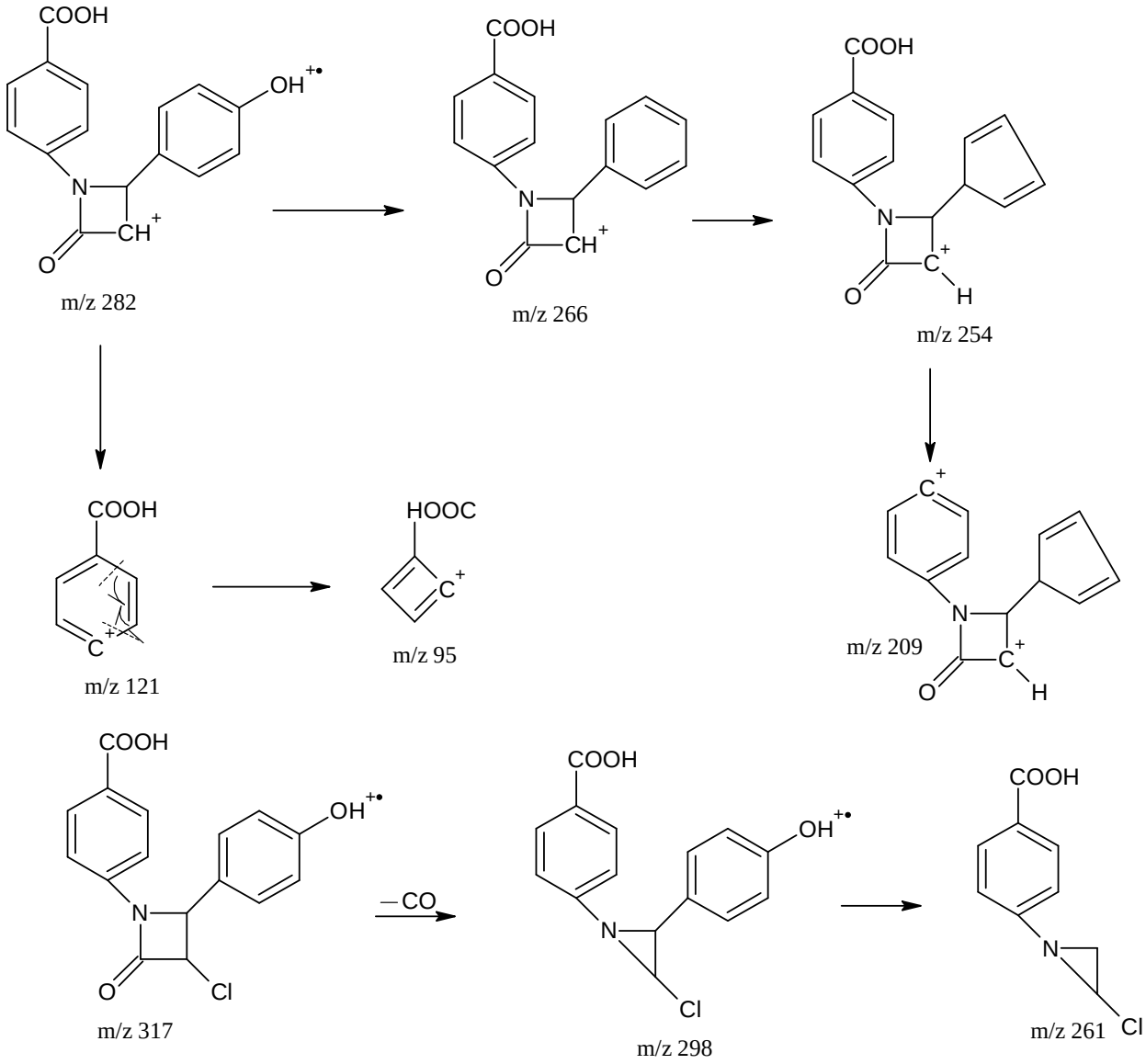


Fig-71: Fragmentation of 4-[3-chloro-2-(4-hydroxyphenyl)-4-oxoazetidin-1-yl] benzoic acid

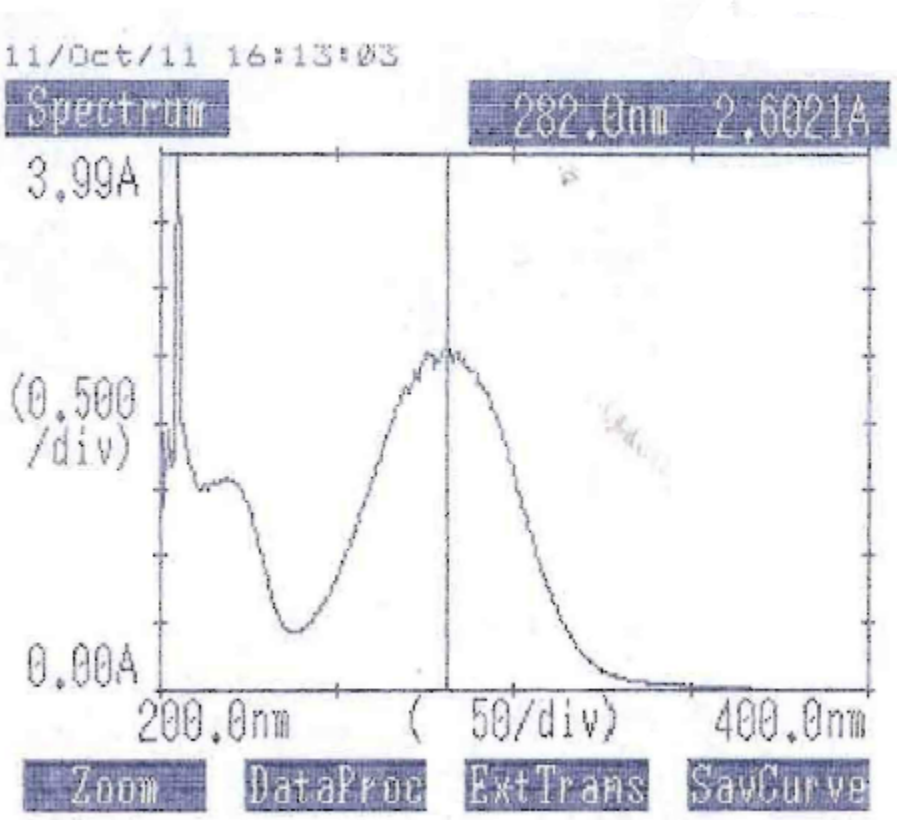


Fig-66: UV Spectrum of compound SS_3

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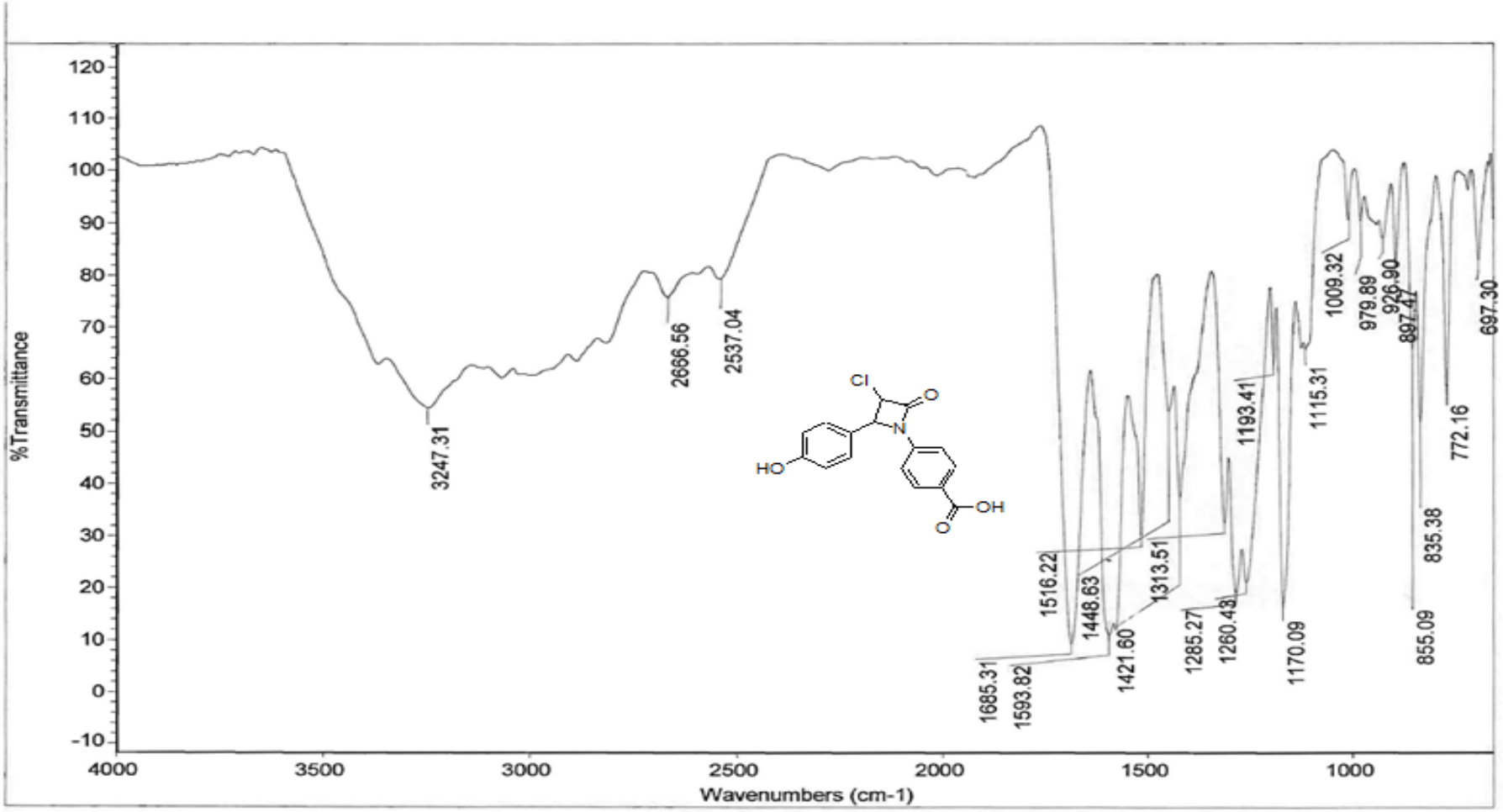


Fig-67: IR Spectrum of compound SS_3

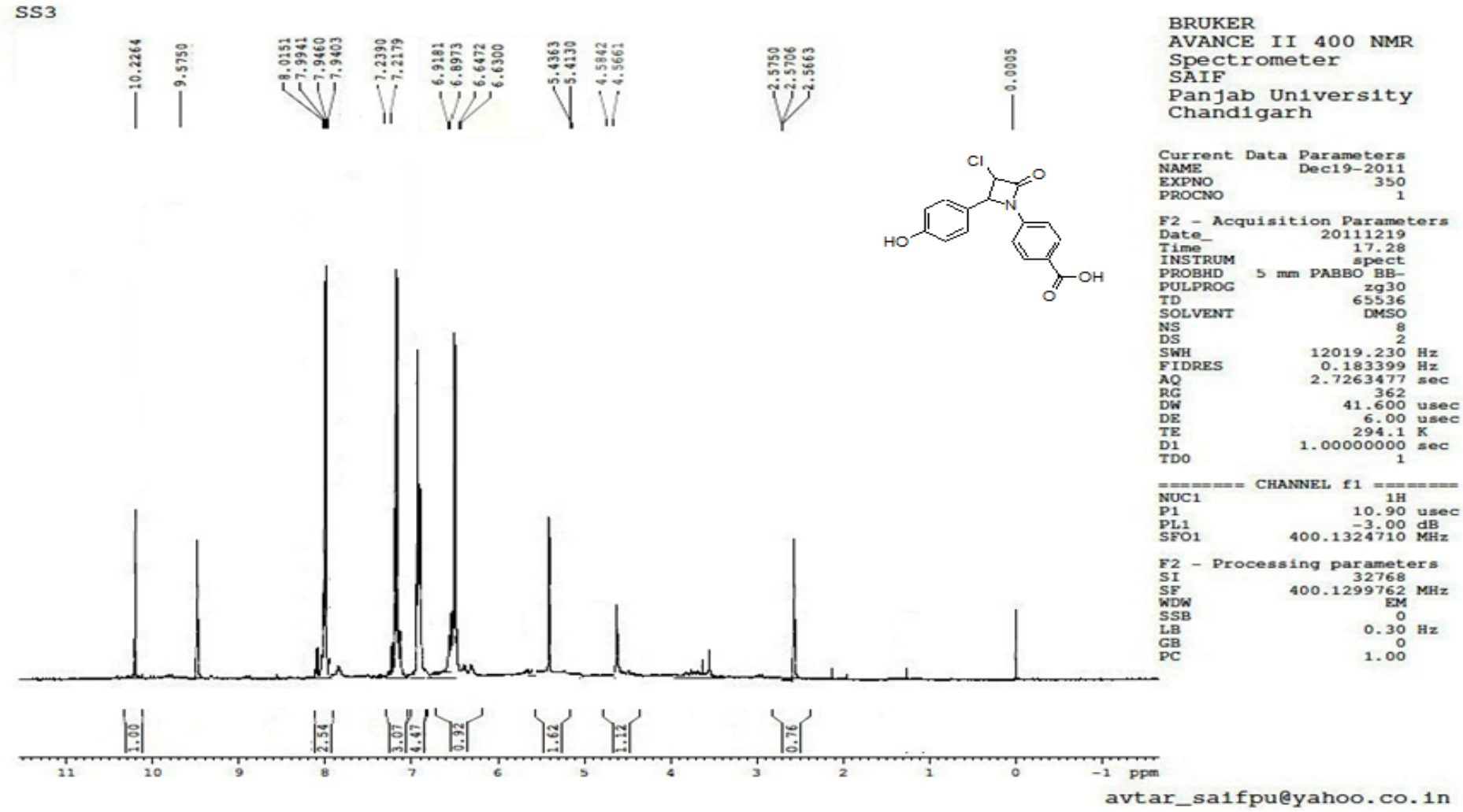


Fig-68: ^{1}H-NMR Spectrum of the compound SS_3

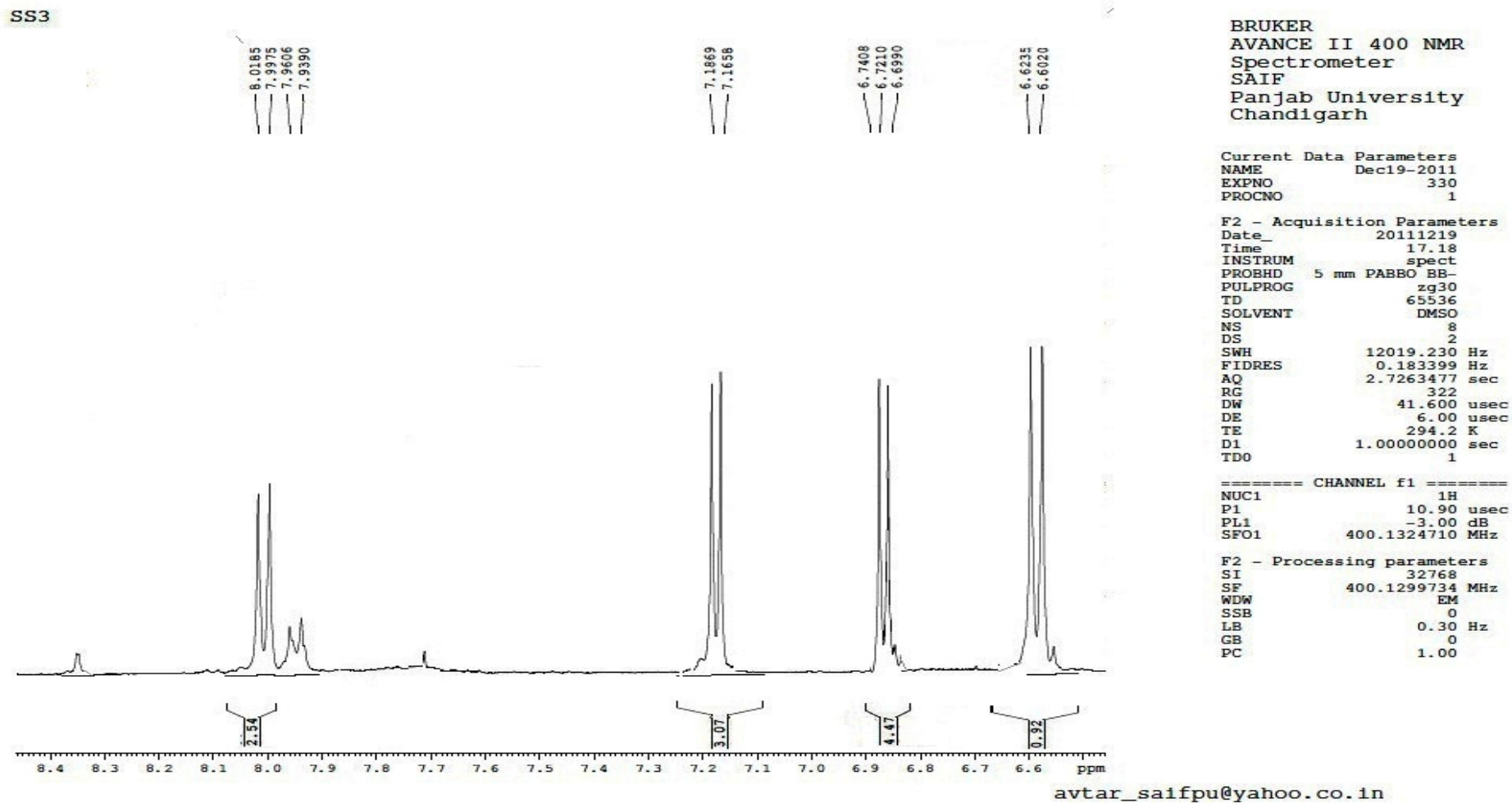


Fig-69: ^{1}H-NMR Spectrum of the compound SS_3(Zoom View 1)

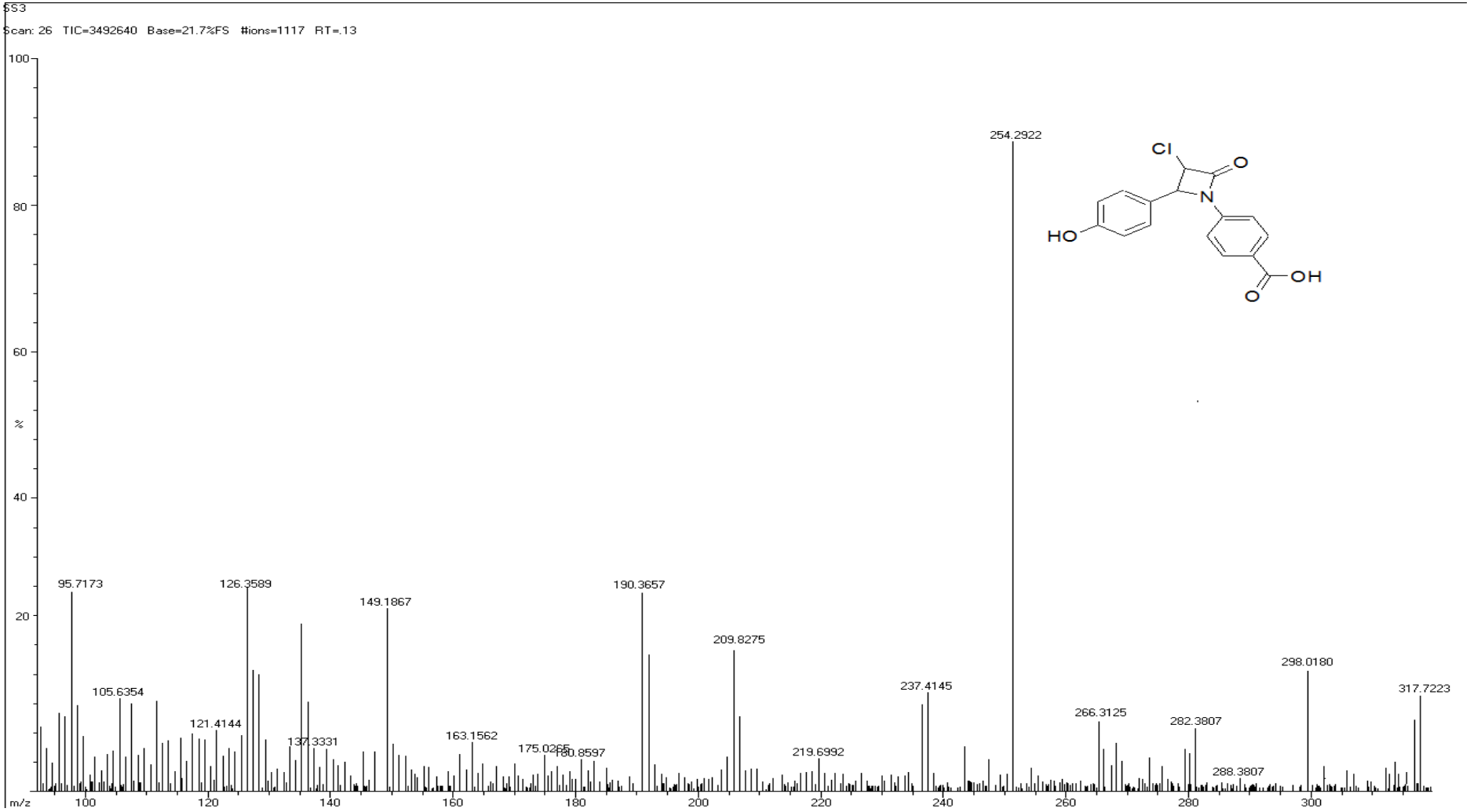


Fig-70: Mass Spectrum of the compound SS_3

6.2.4. Spectral analysis of 4-[2-[4-dimethylaminophenyl]-4-oxo-1,3-thiazolidin-3-yl]-benzoic acid (SS_4)

UV: (Fig-72)

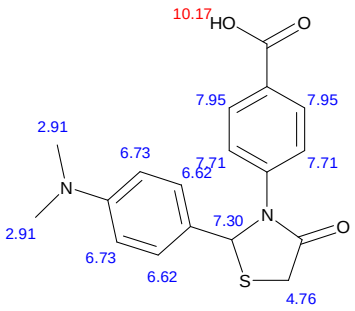
λ_{max} (MeOH) 340.0 (ε_{max} 0.9750)

λ_{max} (MeOH) 270.5 (ε_{max} 1.0425)

IR (KBr): (Fig-73)

Wavelength (cm^{-1})	Assignment
2923.84	Aromatic C-H (stretching)
1658.42	C=O (stretching)
1600.58	Aromatic C=C (stretching)
1456.70	N-CH_3 (stretching)
1437.31	C-O-H (bending)
1362.63	C-N (stretching)
1252.41	C-O (stretching)
820.93	Aromatic C-H (bending)
691.78	C-S (stretching)

NMR (DMSO-d_6): (Fig-74-76)



(8 aromatic protons, 6 methyl protons, 3 cyclic protons and 1 proton on carboxylic acid)

δ	Assignment
10.17	(1H, s, Ar-COOH)
7.95-7.71	(4H, m, Ar-H of benzoic acid)
4.76	(2H, s, CH_2-S)
6.73-6.62	(4H, m, Ar-H of dimethyl amino phenyl group)
7.30	(1H. s, S-CH-N)
2.91	(6H, s, $N(CH_3)_2$)

MASS: (Fig-77)

The structure of the compound was further confirmed by its fragmentation peaks which are as follows:

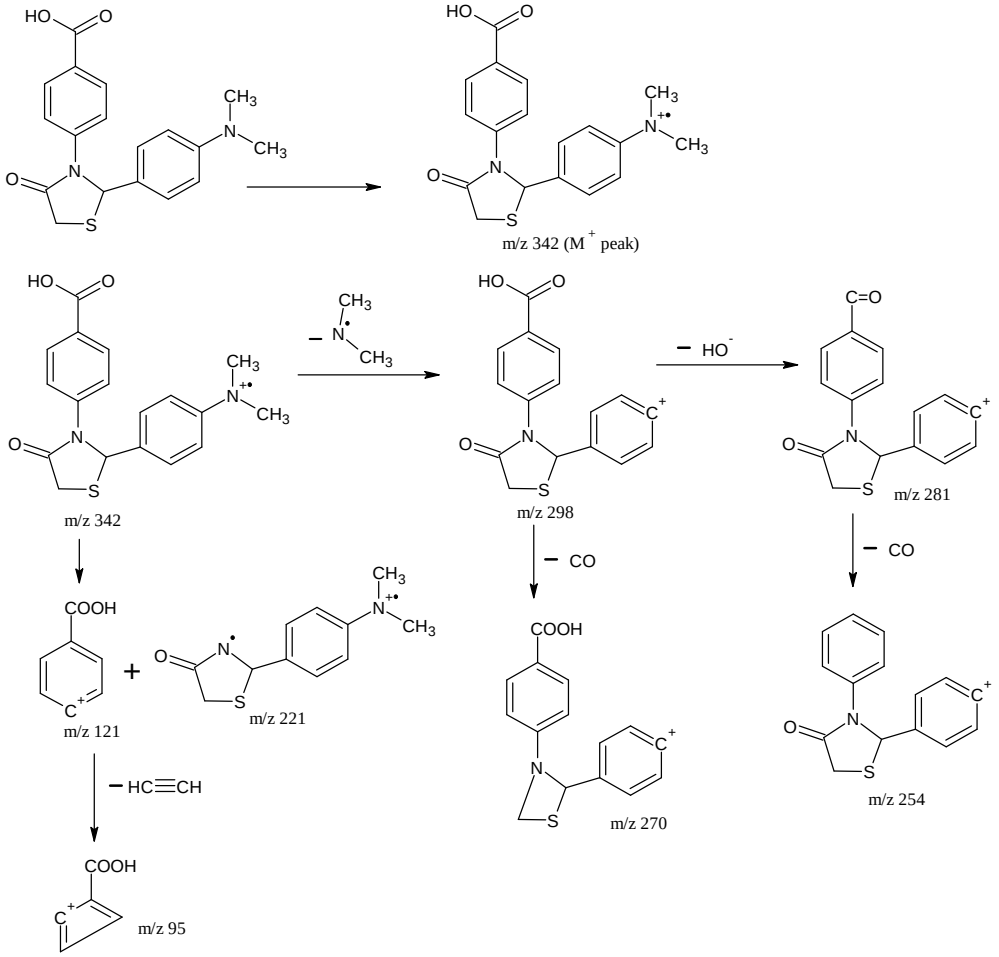


Fig-78: Fragmentation of 4-[2-[4-dimethylaminophenyl]-4-oxo-1,3-thiazolidin-3-yl]benzoic acid

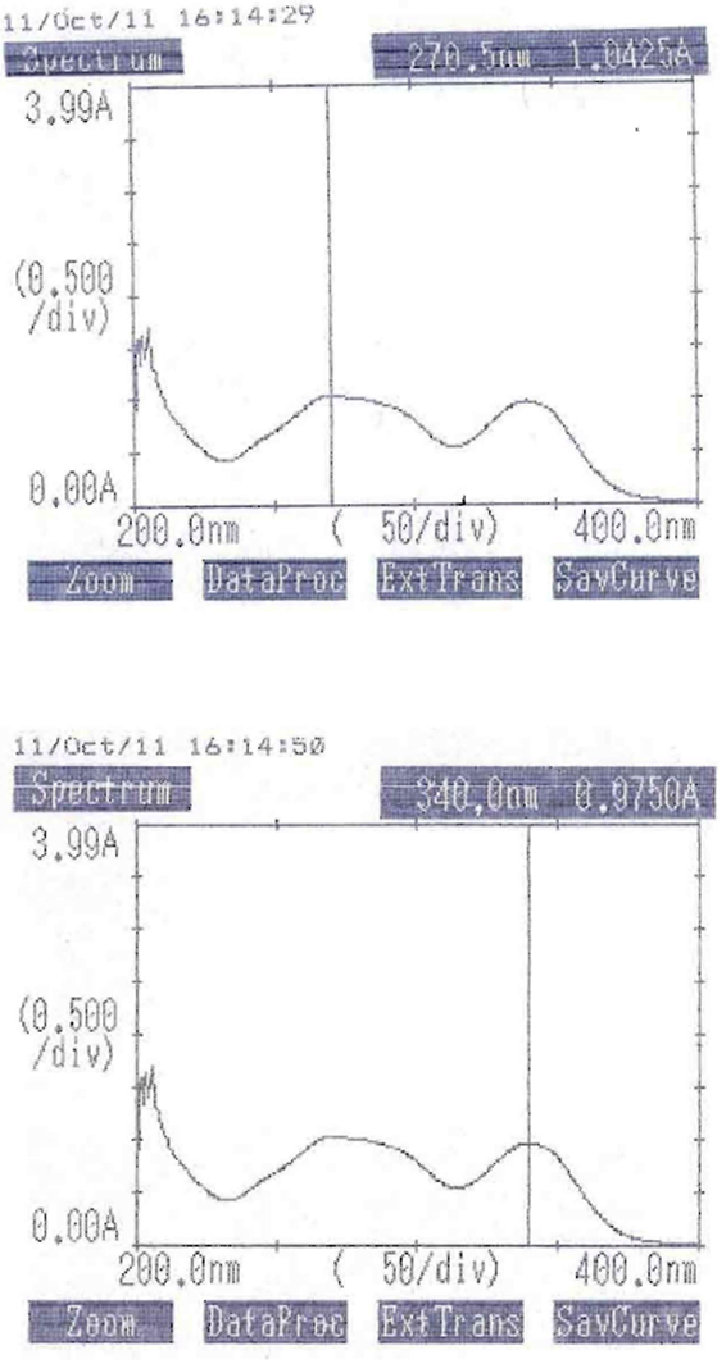


Fig-72: UV Spectrum of compound SS_4

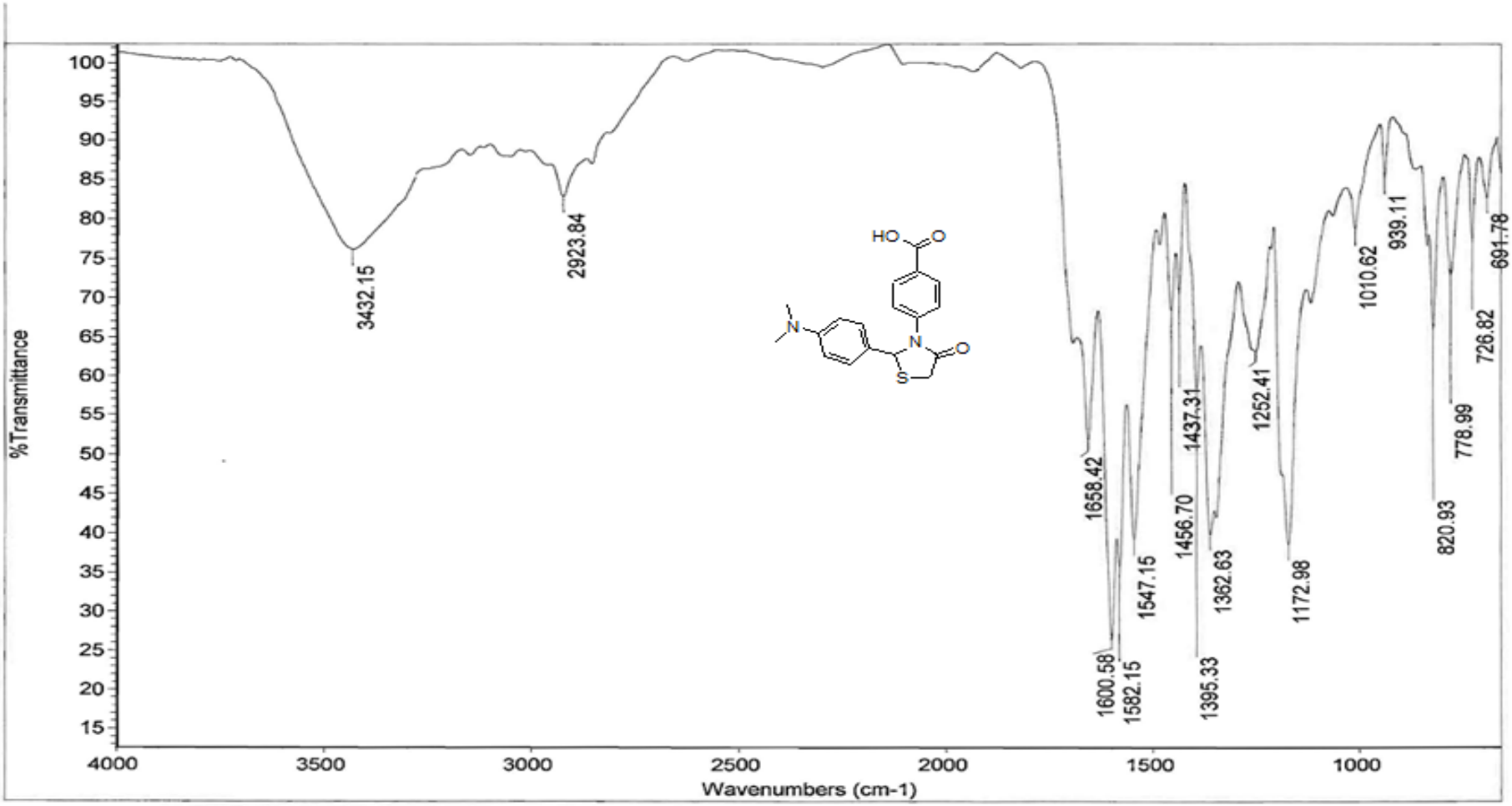


Fig-73: IR Spectrum of compound SS_4

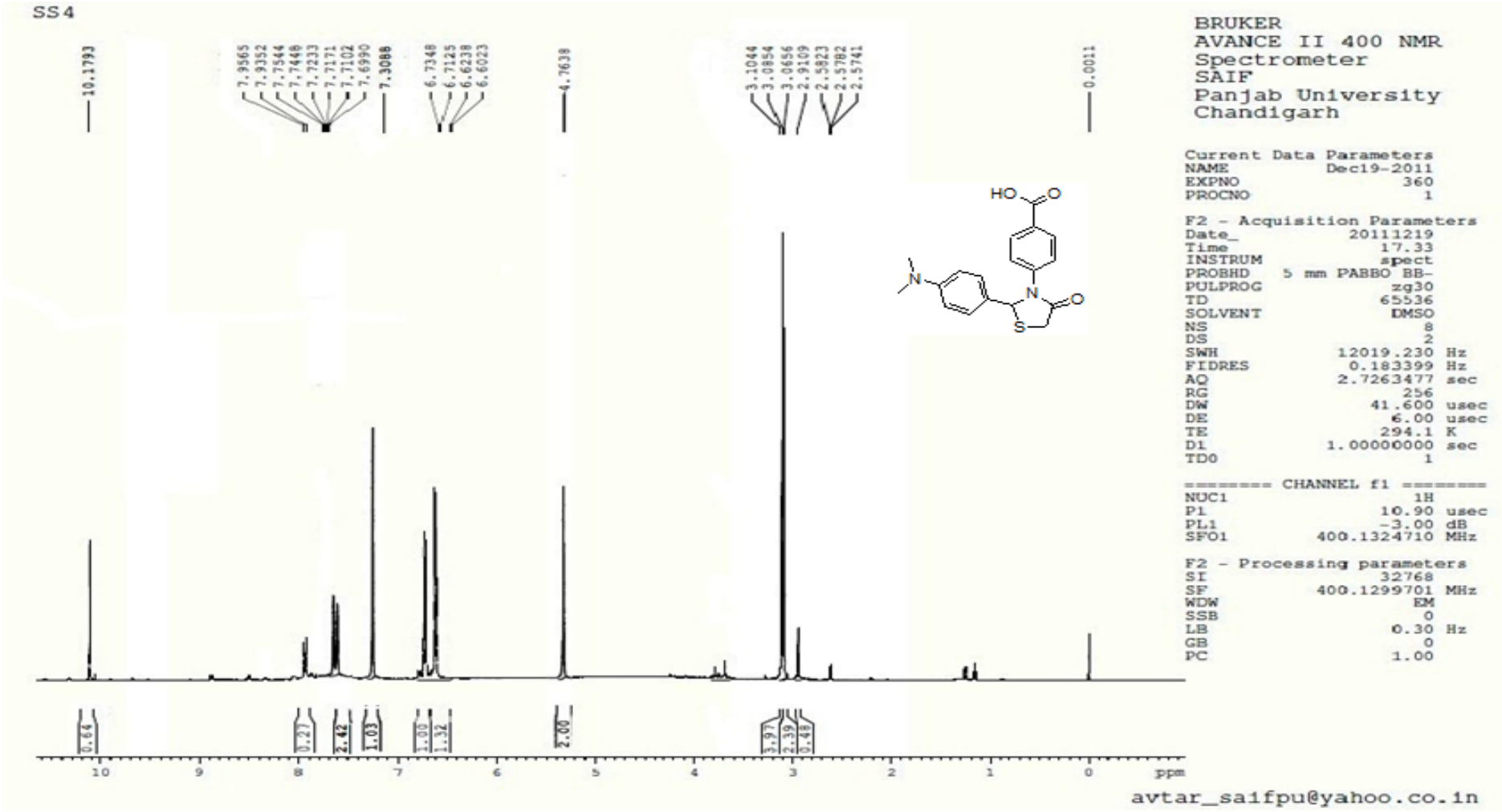


Fig-74: ^{1}H-NMR Spectrum of the compound SS_4

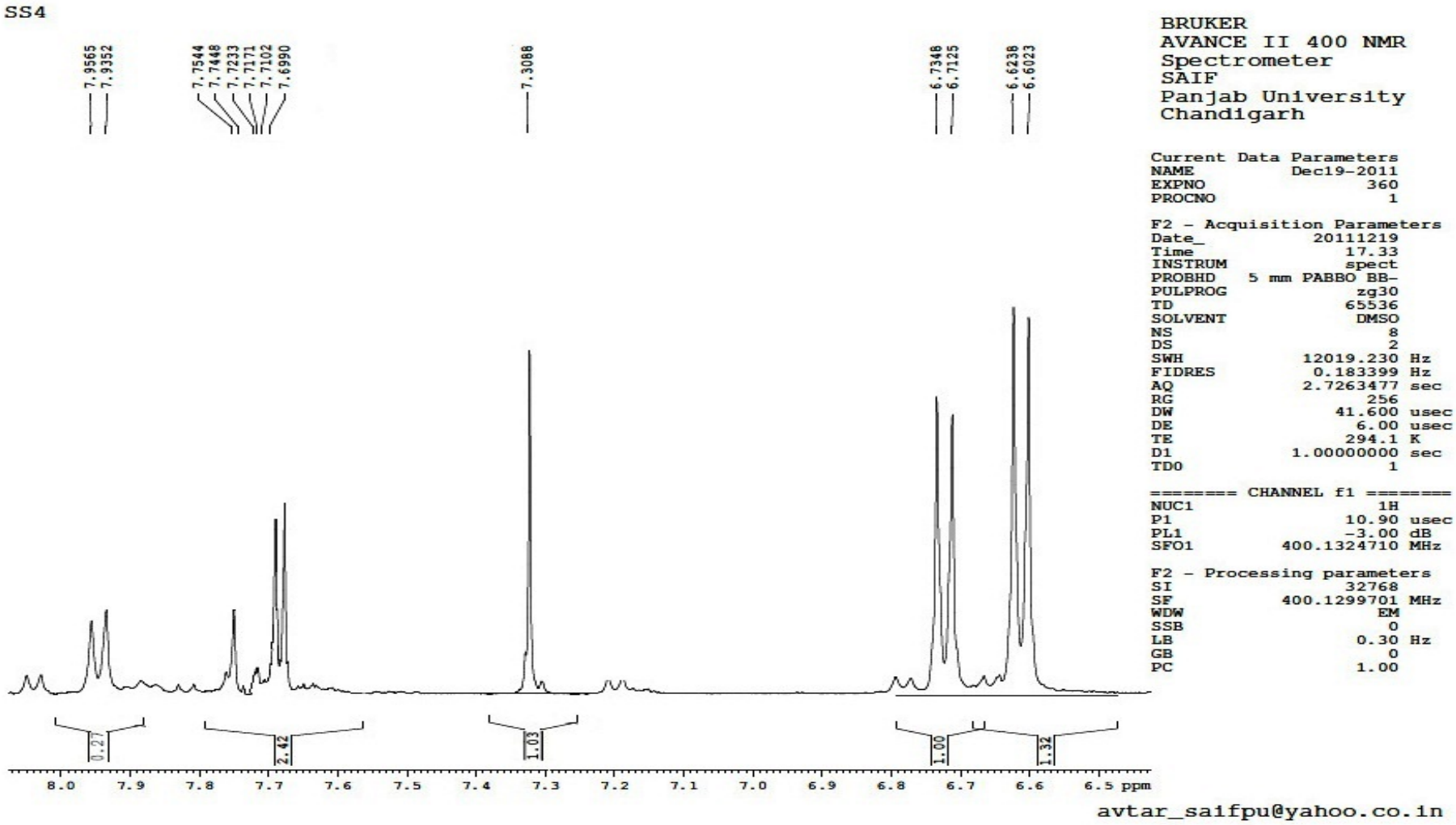


Fig-75: ^{1}H-NMR Spectrum of the compound SS_4(Zoom View 1)

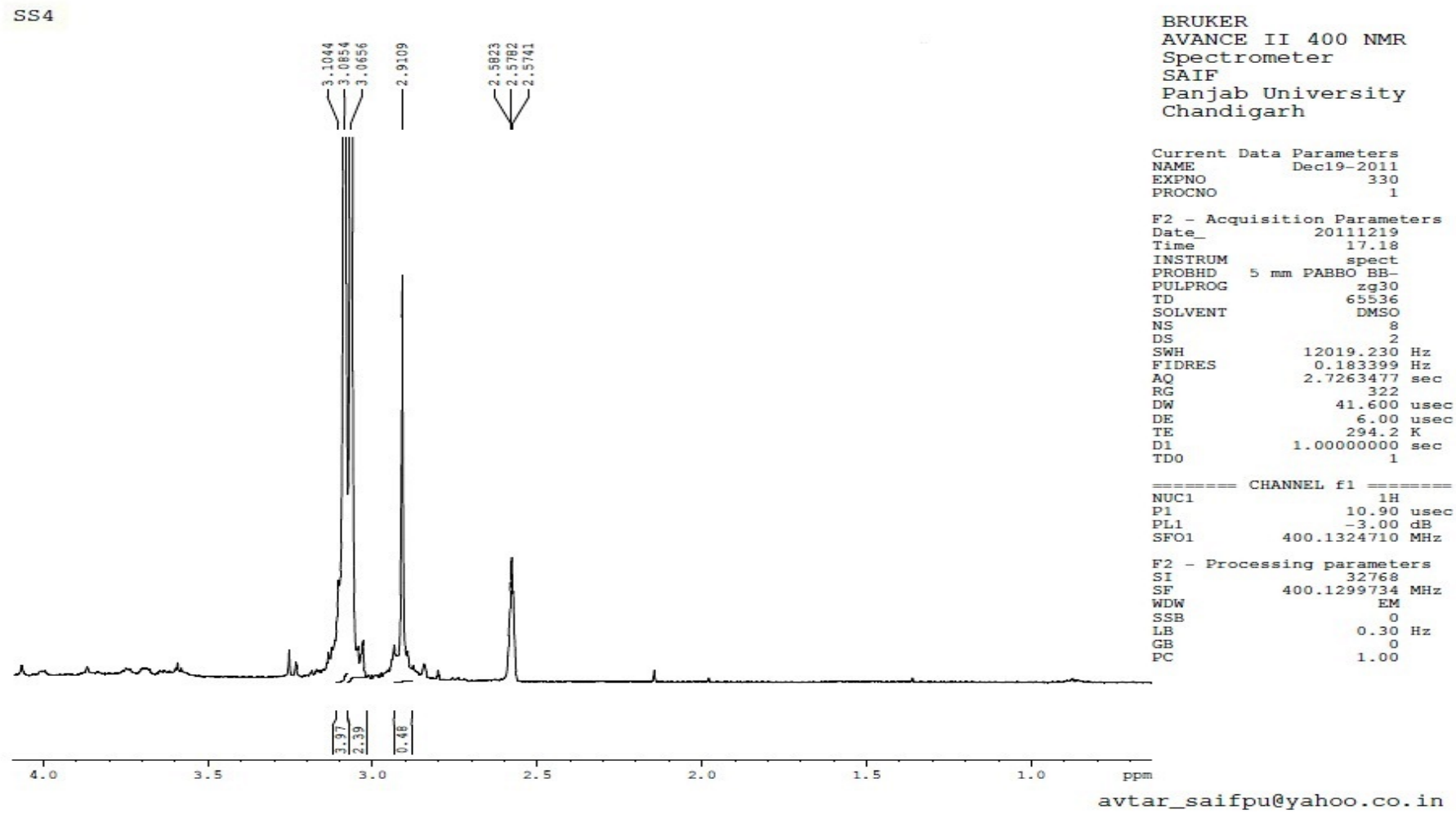


Fig-76: ^{1}H-NMR Spectrum of the compound SS_4(Zoom View 2)

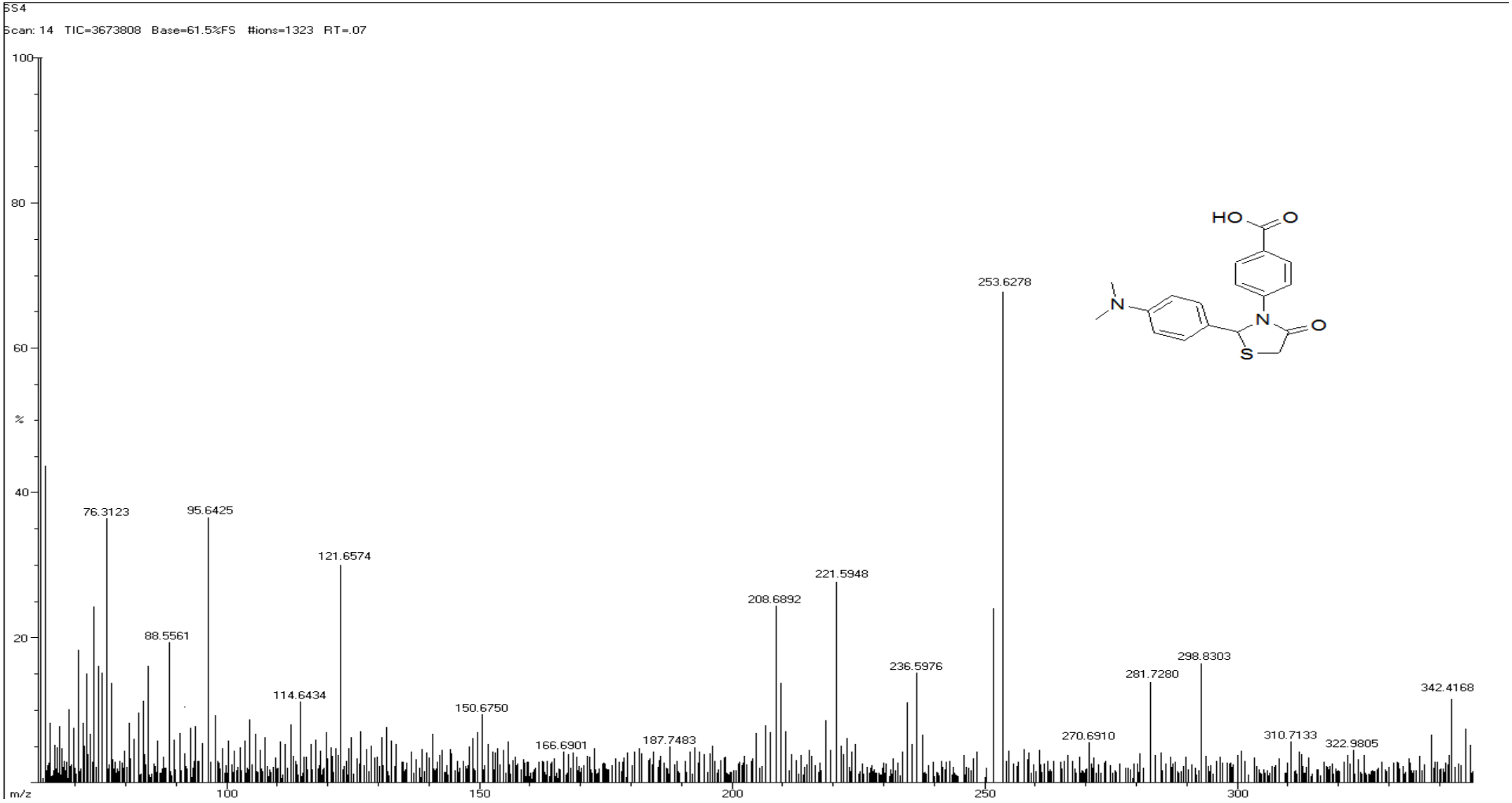


Fig-77: Mass Spectrum of the compound SS_4

6.2.5. Spectral analysis of 4-{2-[4-nitrophenyl]-4-oxo-1,3-thiazolidin-3-yl}-benzoic acid (SS_5)

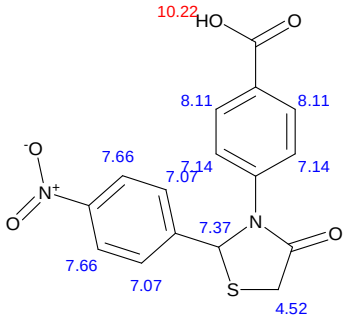
UV: (Fig-79)

λ_{max} (MeOH) 290.0 (ε_{max} 0.9673)

IR (KBr): (Fig-80)

Wavelength (cm^{-1})	**Assignment**
3106.97	Aromatic C-H (stretching)
1679.56	C=O (stretching)
1597.47	Aromatic C=C (stretching)
1517.93	Aromatic NO_2 asymmetric (stretching)
1408.50	C-O-H (bending)
1347.18	C-N (stretching)
1316.19	Aromatic NO_2 symmetric (stretching)
1247.15	C-O (stretching)
854.70	Aromatic C-H (bending)
698.33	C-S (stretching)

NMR (DMSO-d_6): (Fig-81 & 82)



(8 aromatic protons, 3 cyclic protons and 1 proton on carboxylic acid)

δ	Assignment
10.22	(1H, s, Ar-COOH)
8.11-7.66	(8H, m, Ar-H)
7.37	(1H. s, S-CH-N)
4.52	(2H, s, CH_2-S)

MASS: (Fig-83)

The structure of the compound was further confirmed by its fragmentation peaks which are as follows:

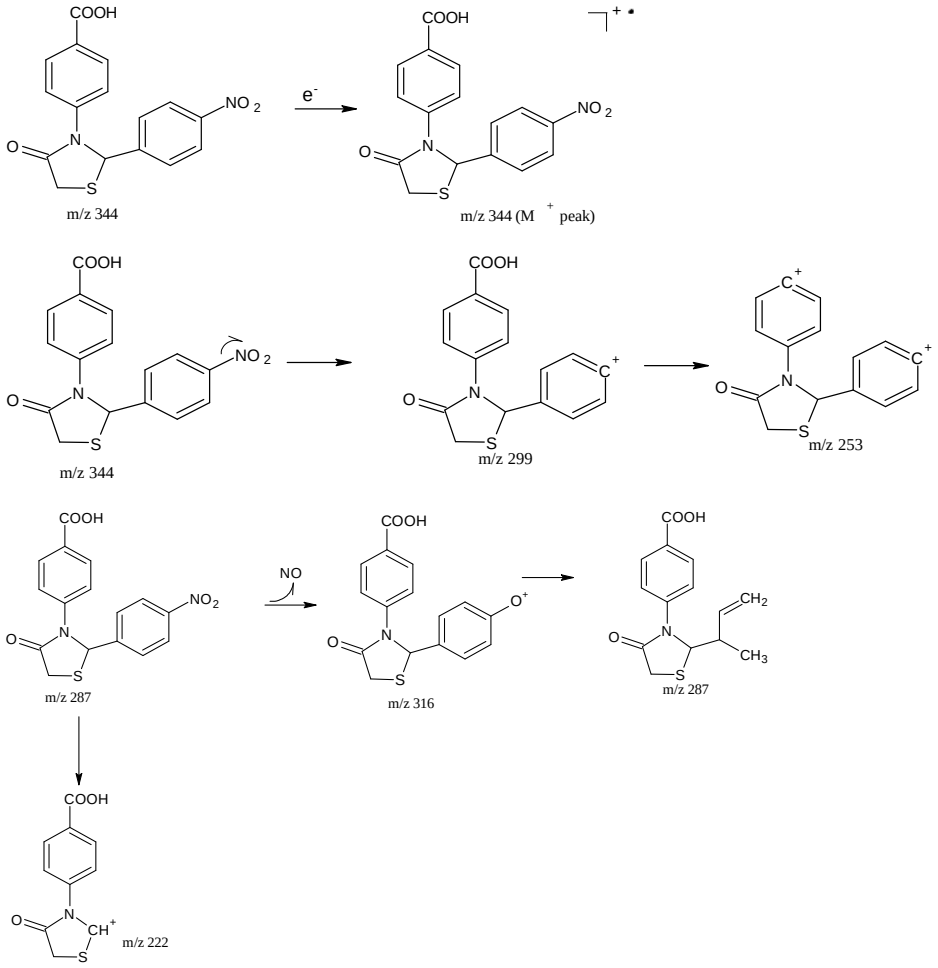


Fig-84: Fragmentation of 4-{2-[4-nitrophenyl]-4-oxo-1,3-thiazolidin-3-yl} benzoic acid

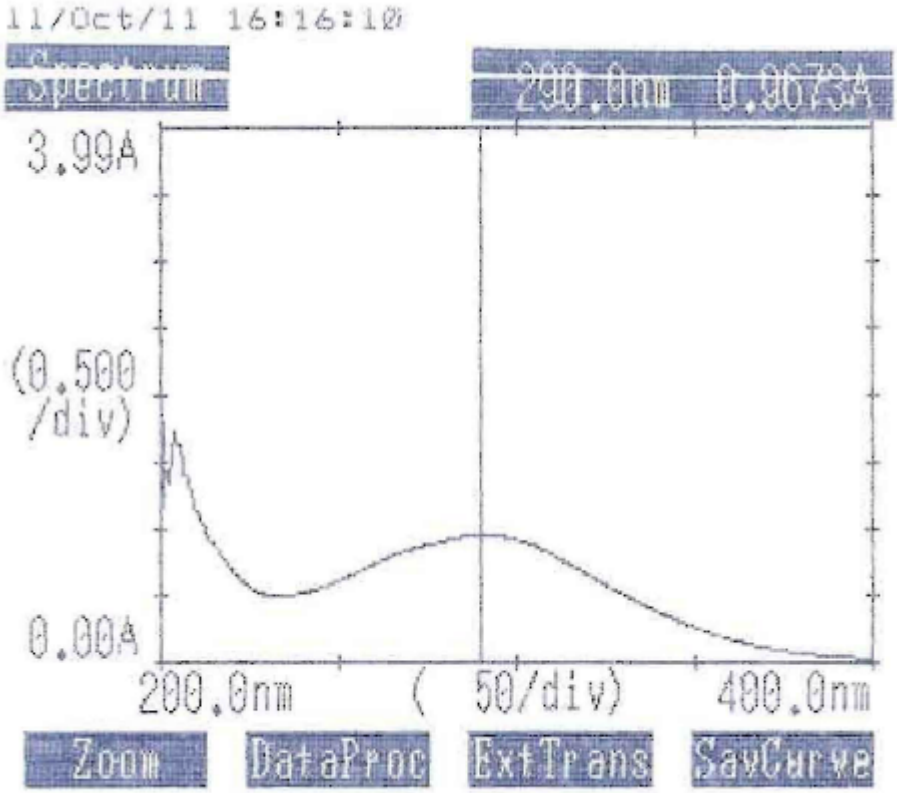


Fig-79: UV Spectrum of compound SS_5

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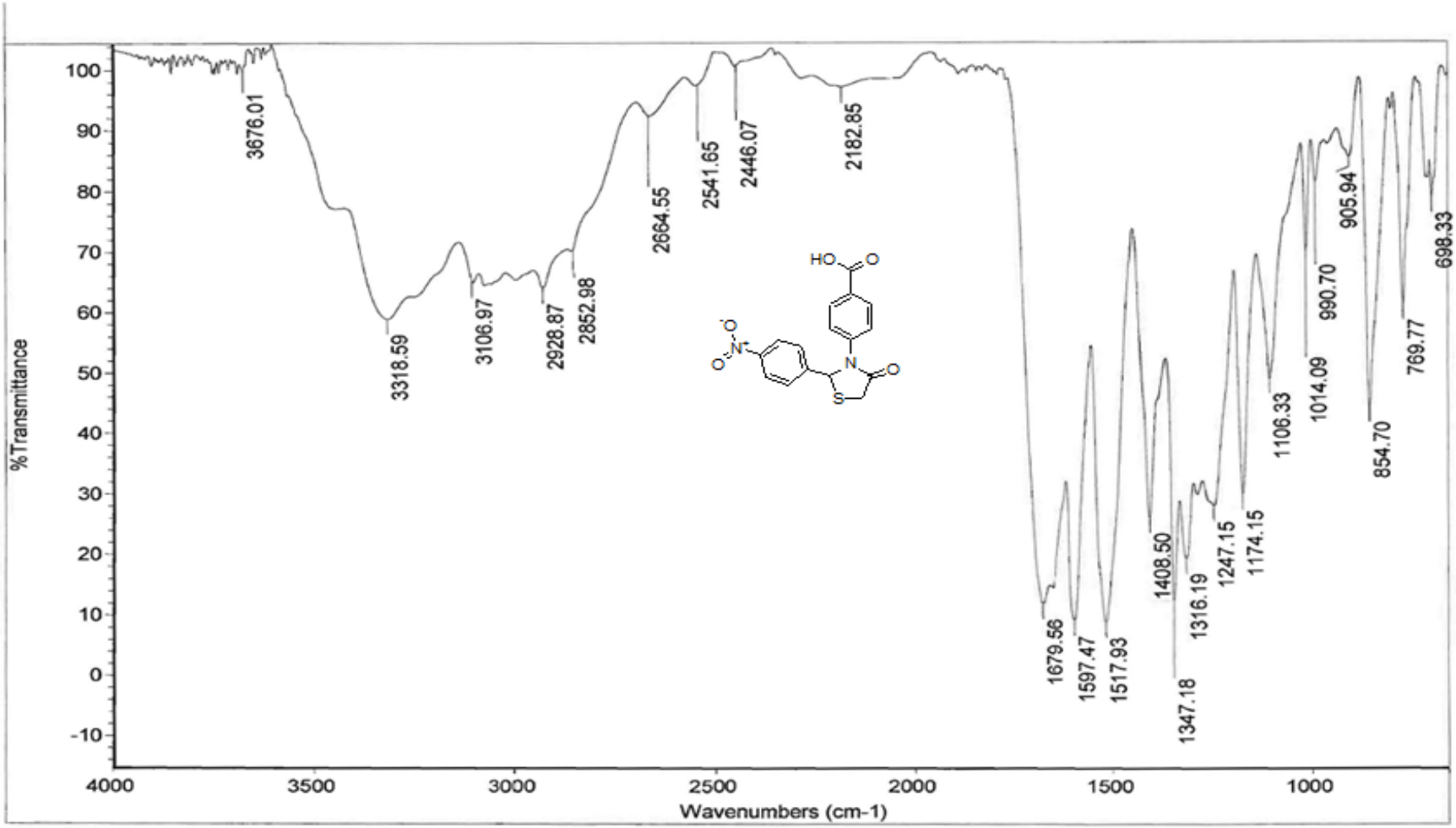


Fig-80: IR Spectrum of compound SS_5

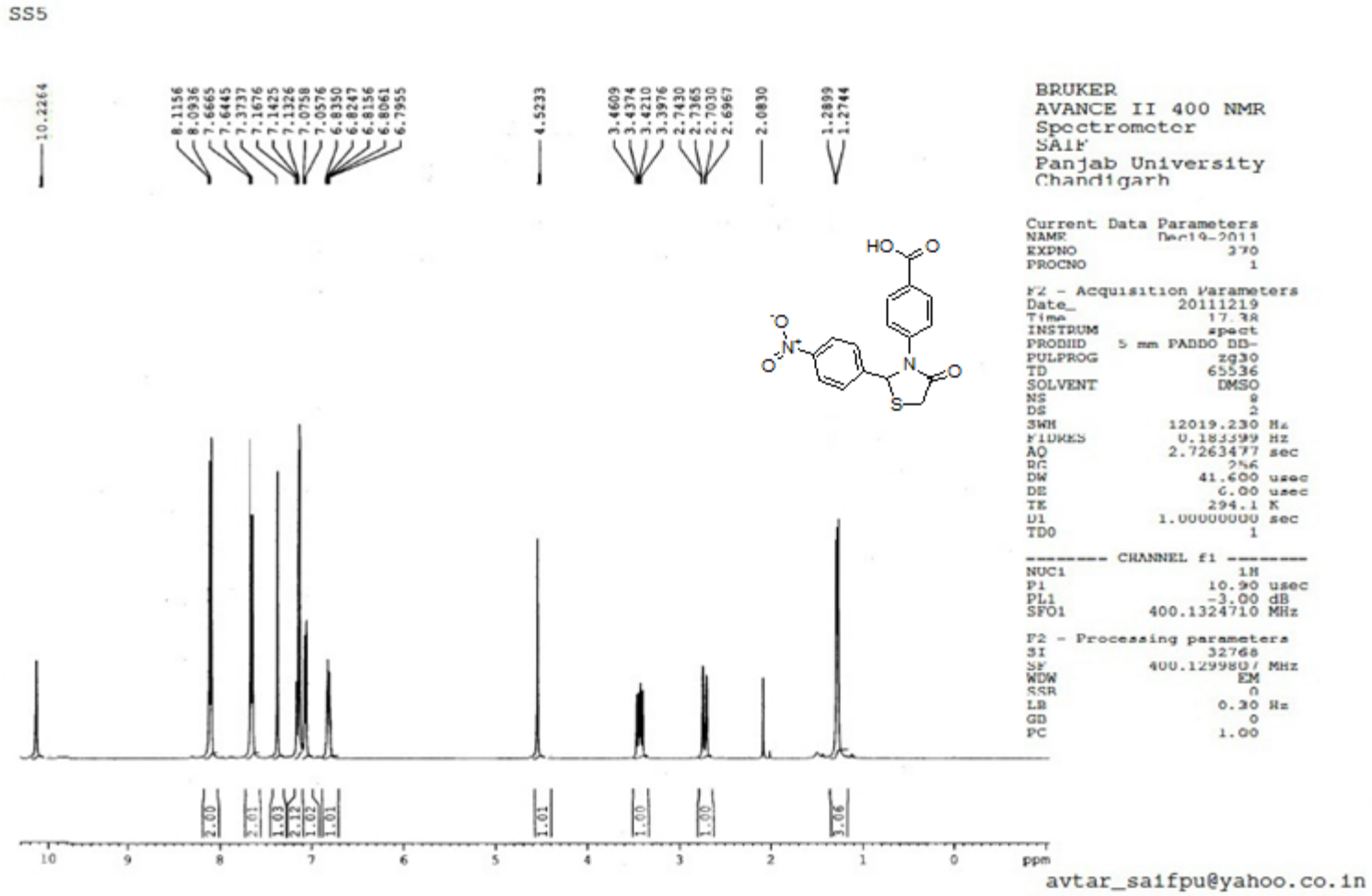


Fig-81: ^{1}H-NMR Spectrum of the compound SS_5

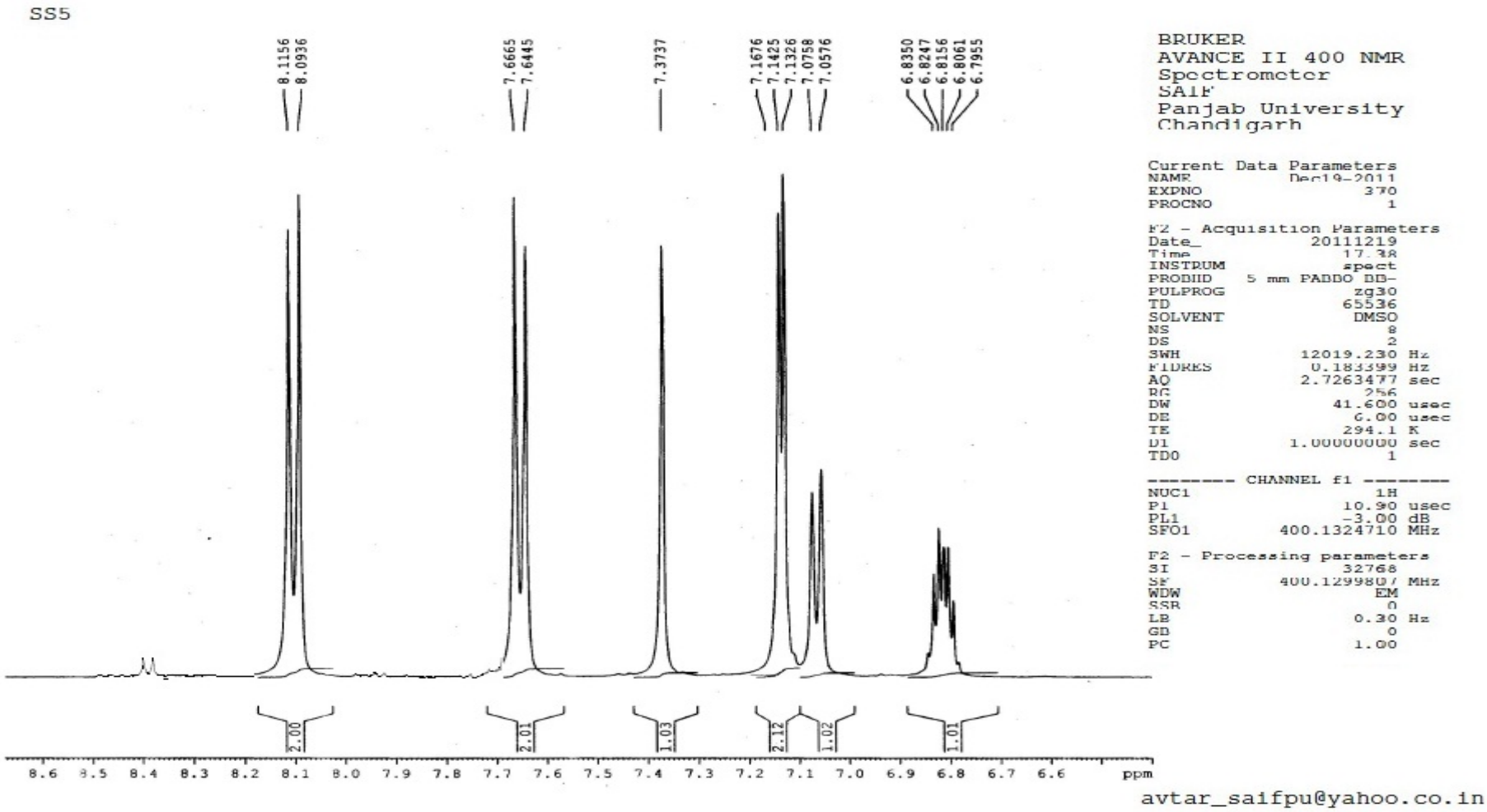


Fig-82: ^{1}H-NMR Spectrum of the compound SS_5(Zoom View 1)

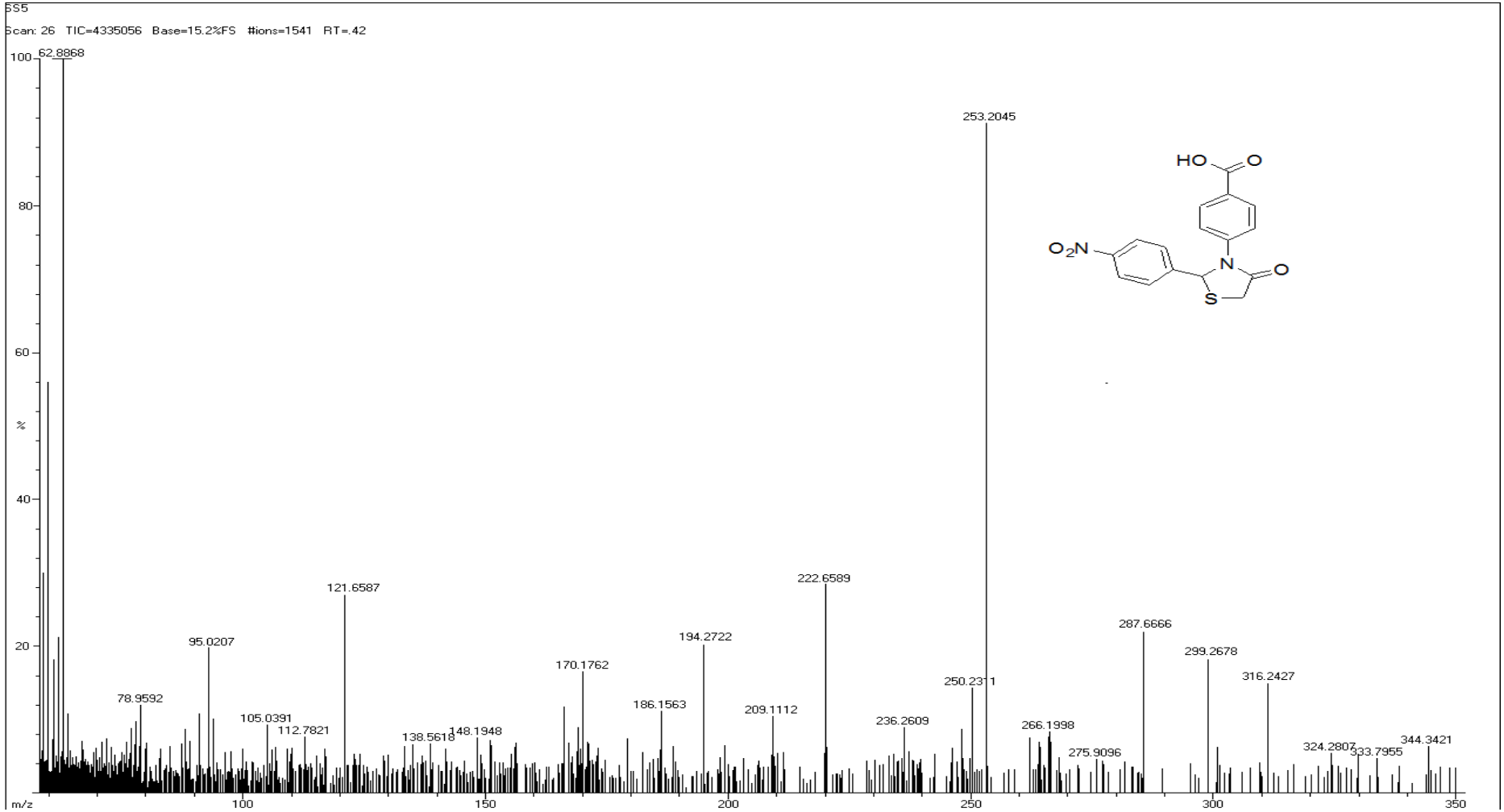


Fig-83: Mass Spectrum of the compound SS_5

6.2.6. Spectral analysis of 4-{2-[4-hydroxyphenyl]-4-oxo-1,3-thiazolidin-3-yl}-benzoic acid (SS_6)

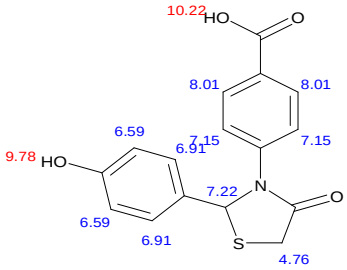
UV: (Fig-85)

λ_{max} (MeOH) 264.5 (ε_{max} 1.6844)

IR (KBr): (Fig-86)

Wavelength (cm^{-1})	**Assignment**
3309.29	OH (stretching)
2922.65	Aromatic C-H (stretching)
1686.48	C=O (stretching)
1593.80	Aromatic C=C (stretching)
1421.46	C-O-H (bending)
1315.45	C-N (stretching)
1282.92	OH (bending)
1254.86	C-O (stretching)
836.03	Aromatic C-H (bending)
691.88	C-S (stretching)

NMR (DMSO-d_6): (Fig-87-89)

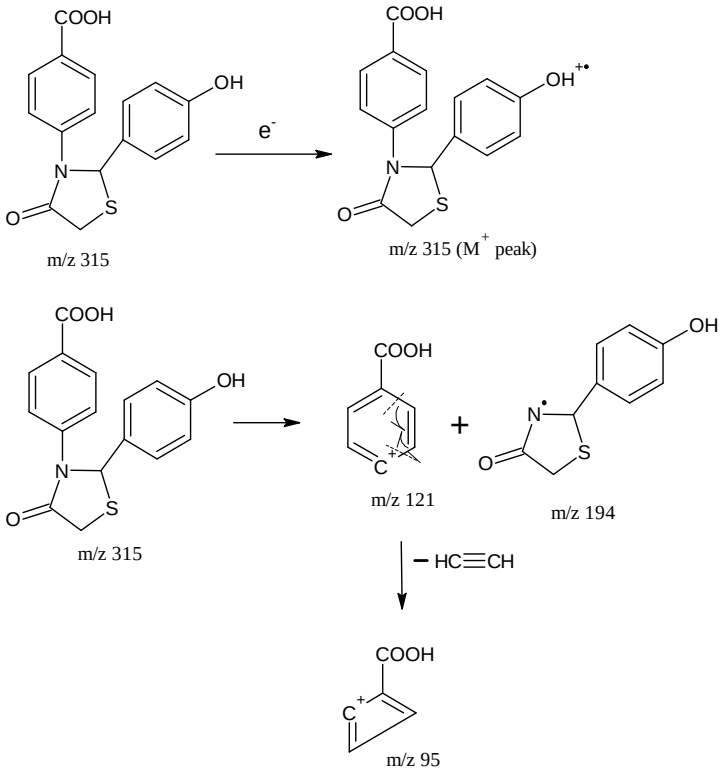


(8 aromatic protons, 3 cyclic protons, one hydroxyl proton and one proton on carboxylic acid)

δ	Assignment
10.22	(1H, s, Ar-COOH)
9.78	(1H, s, Ar-OH)
8.01-7.15	(4H, m, Ar-H of benzoic acid)
7.22	(1H. s, S-CH-N)
6.91-6.59	(4H, m, Ar-H of hydroxyl phenyl group)
4.76	(2H, s, CH_2-S)

MASS: (Fig-90)

The structure of the compound was further confirmed by its fragmentation peaks which are as follows:



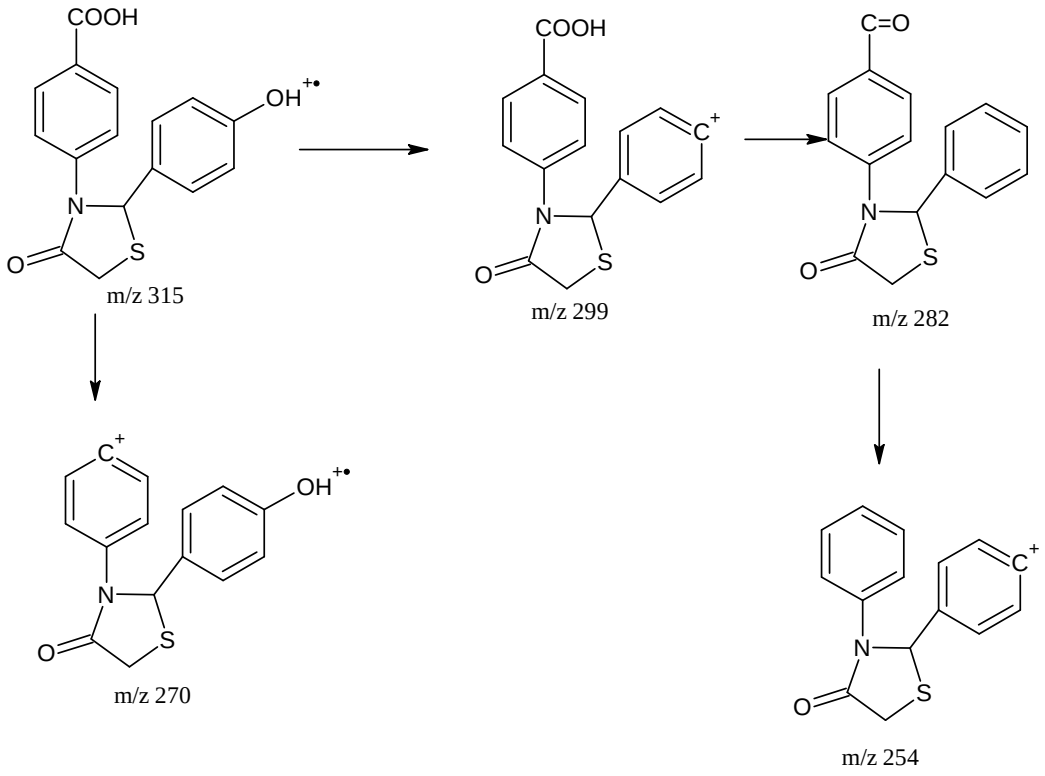


Fig-91: Fragmentation of 4-{2-[4-hydroxyphenyl]-4-oxo-1,3-thiazolidin-3-yl}benzoic acid

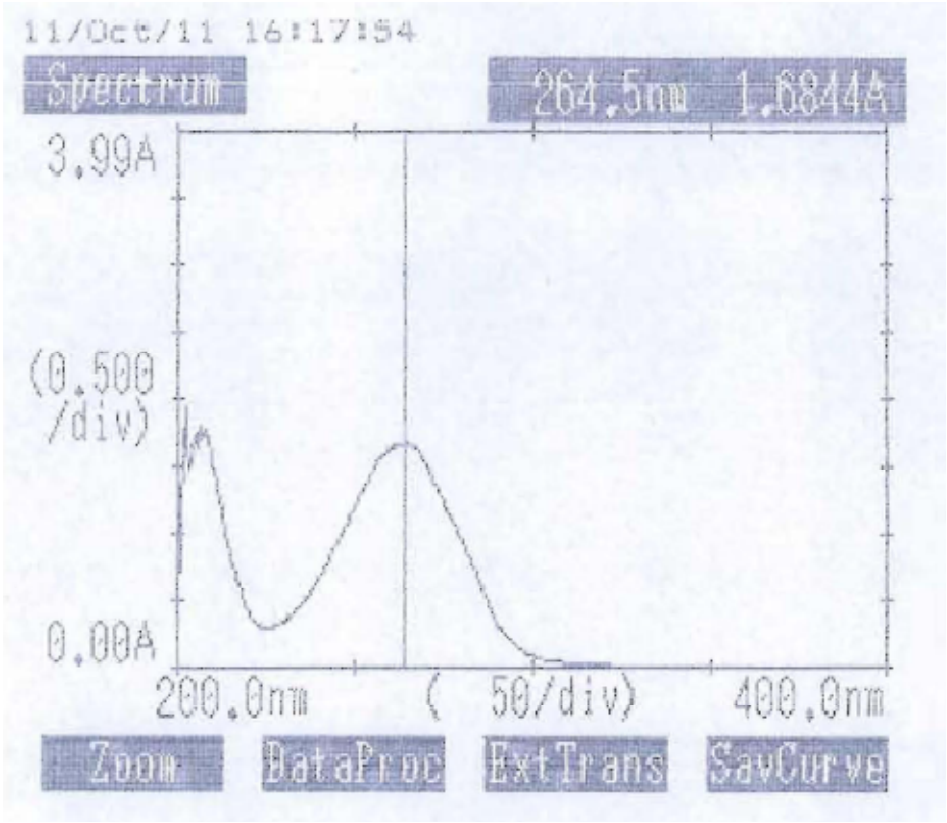


Fig-85: UV Spectrum of compound SS_6

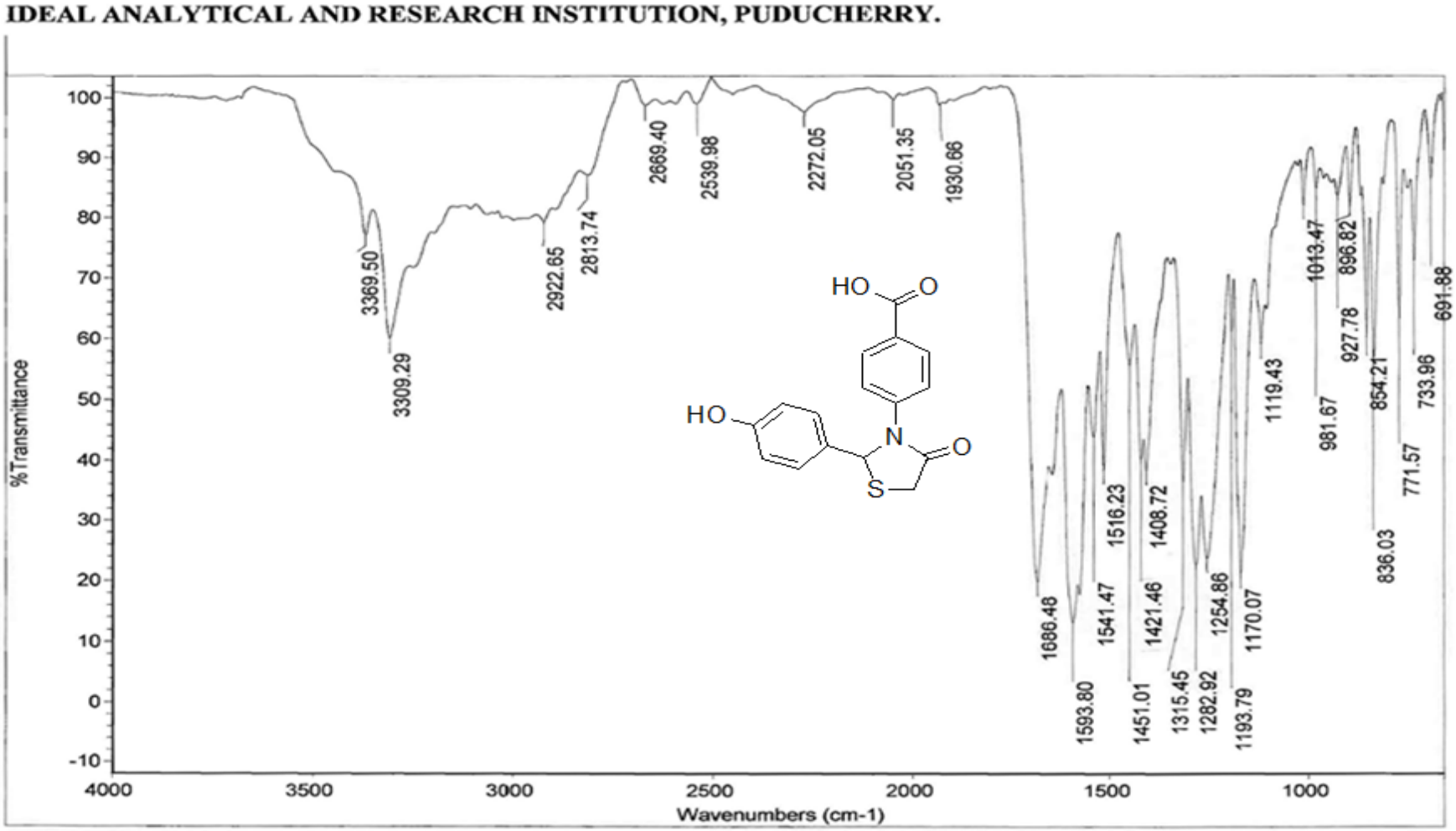


Fig-86: IR Spectrum of compound SS_6

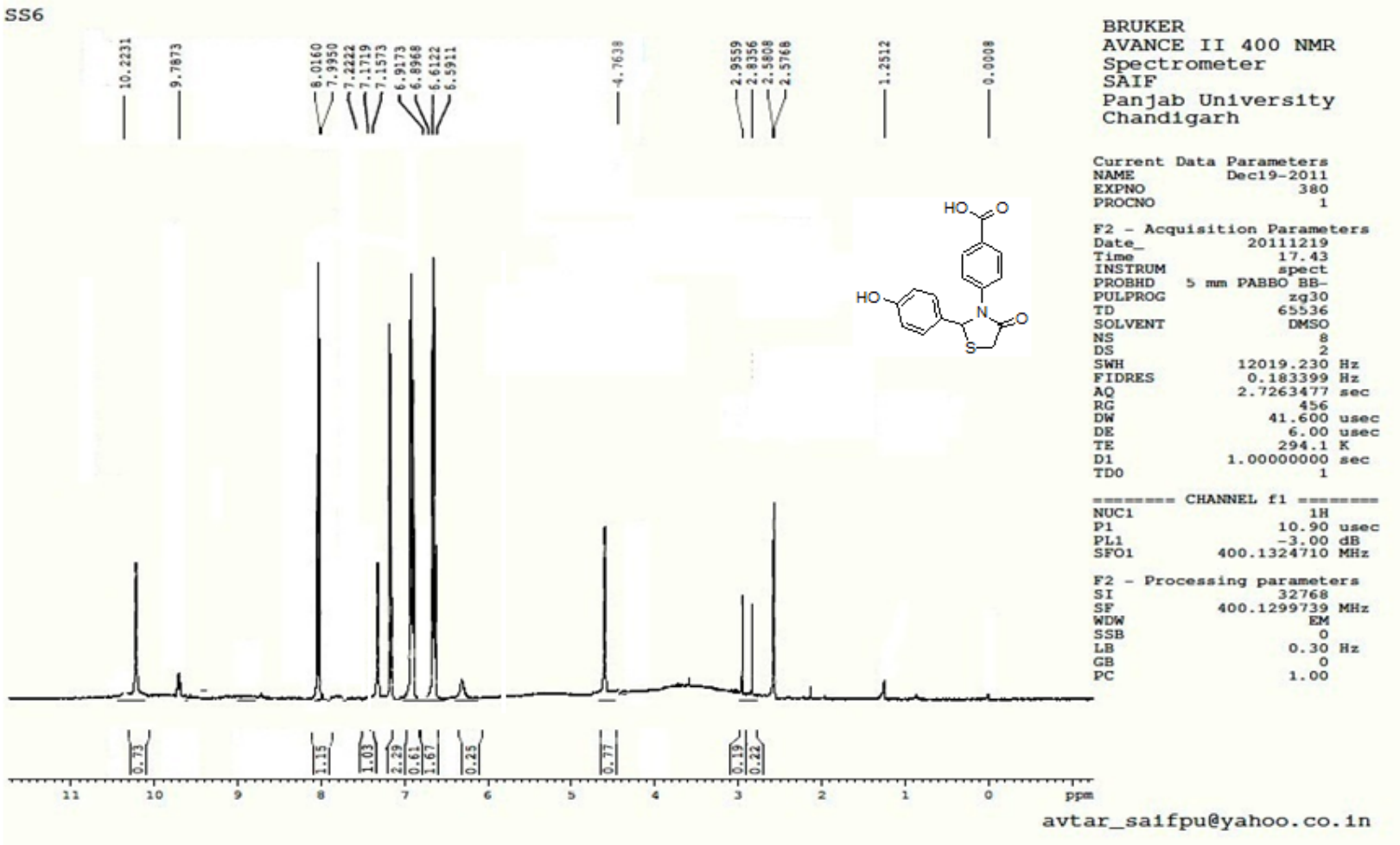


Fig-87: ^{1}H-NMR Spectrum of the compound SS_6

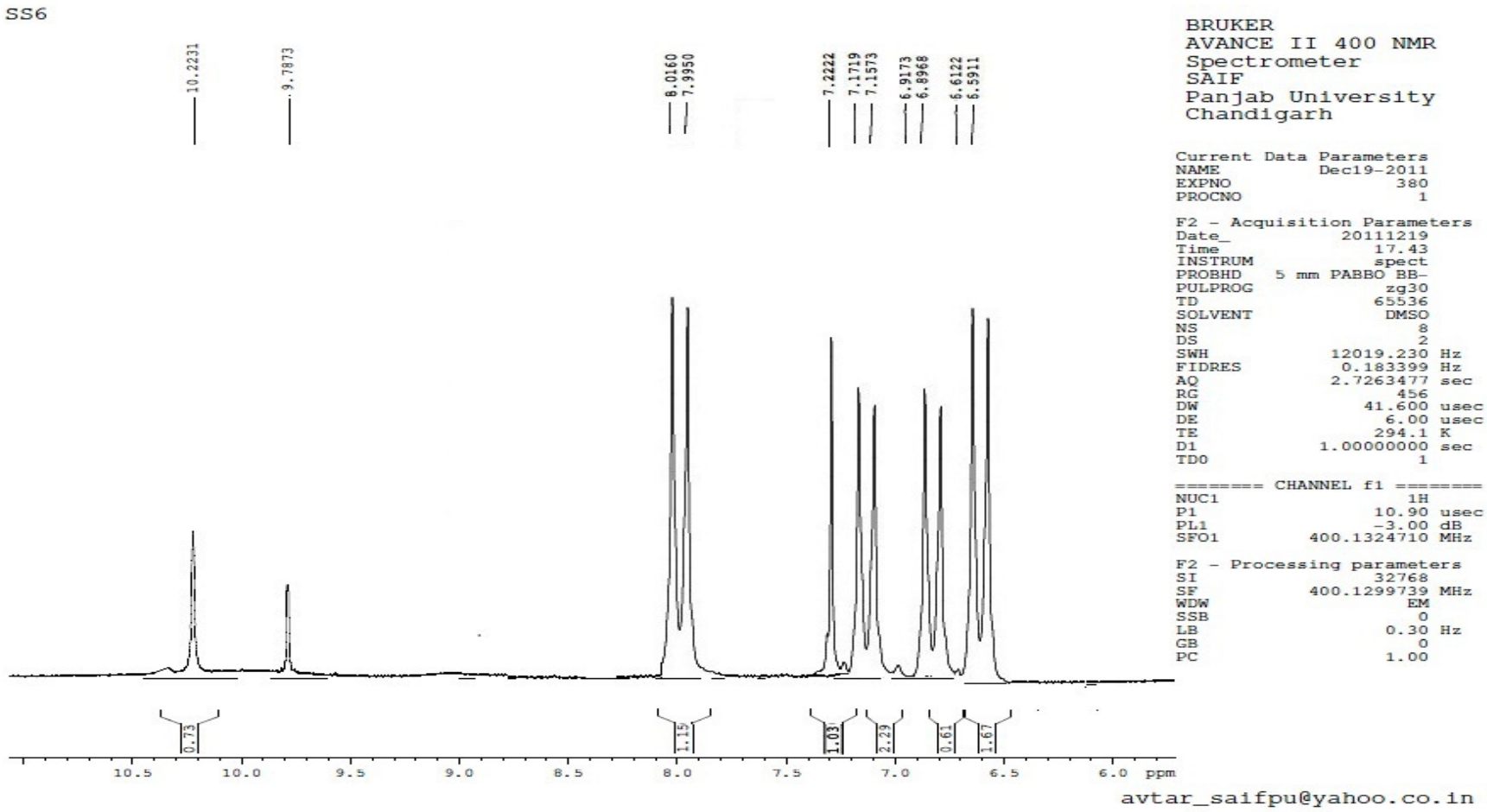


Fig-88: ^{1}H-NMR Spectrum of the compound SS_6(Zoom View 1)

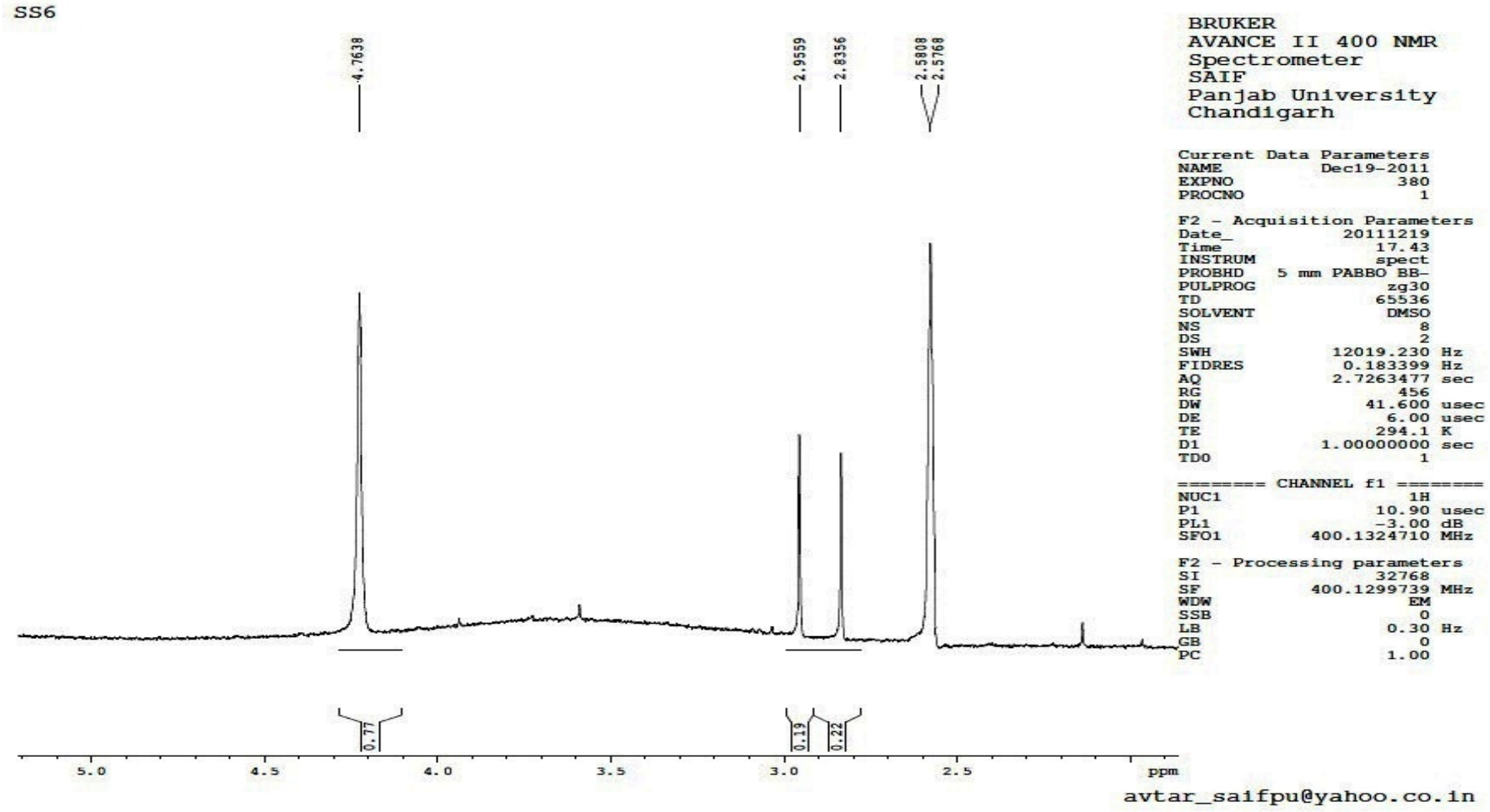


Fig-89: ^{1}H-NMR Spectrum of the compound SS_6(Zoom View 2)

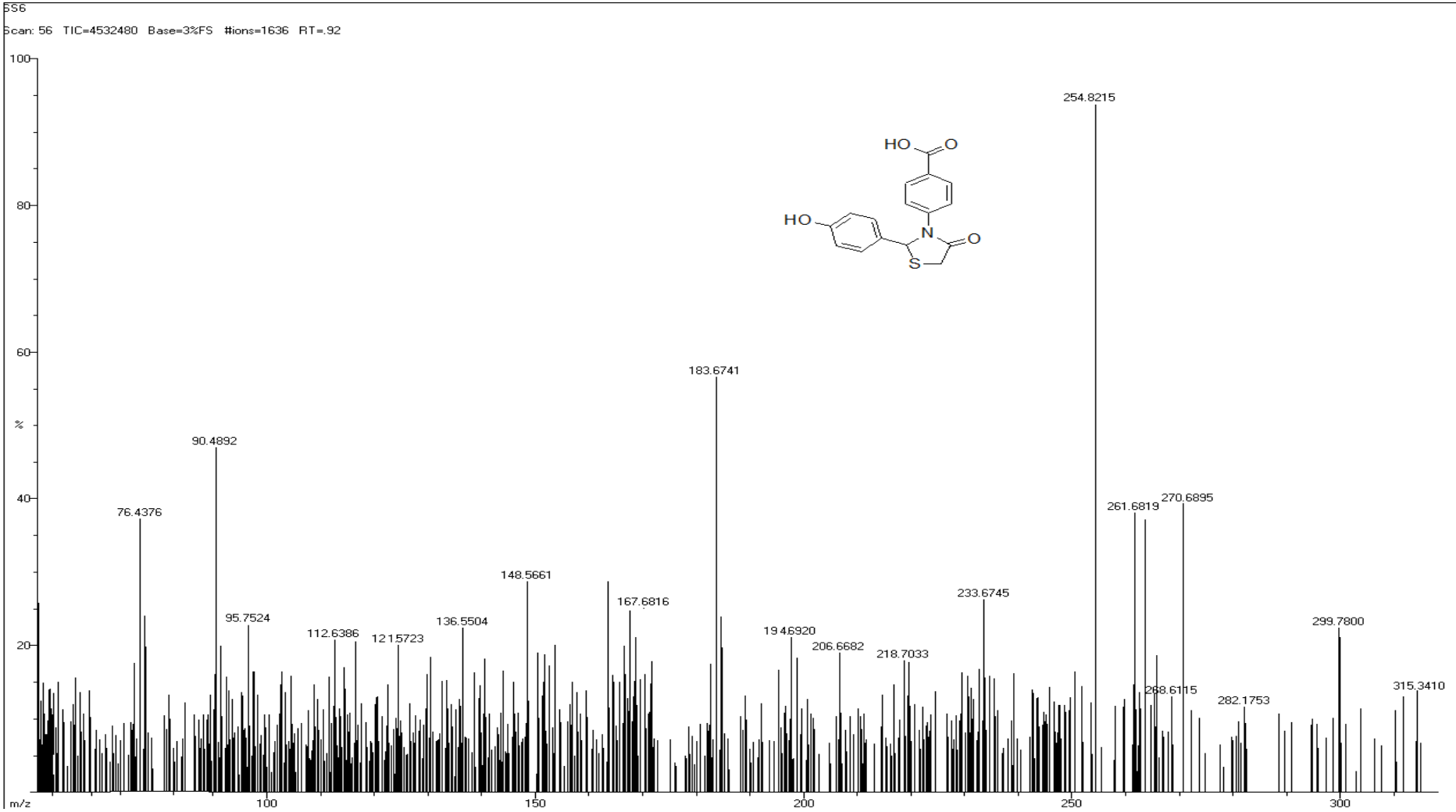


Fig-90: Mass Spectrum of the compound SS_6

6.3- Table 1: Physical and Analytical Data of the Synthesized Compounds

Code No.	Name	Nature	Solubility	Molecular Weight (g)	Molecular Formula	Melting Point (°C)	Percentage Yield (%)	R_f Values (C_6H_5: C_3H_6O)9:1
SB_1	4-(4-dimethylamino benzylideneamino)benzoic acid	Reddish brown amorphous powder	Soluble: Methanol, ethanol, dimethyl formamide. Slightly soluble: Benzene, isopropyl alcohol. Insoluble :Water	268.31	$C_{16}H_{16}N_2O_2$	201	80.37	0.71
SB_2	4-(4-nitrobenzylideneamino)benzoic acid	Yellowish amorphous powder	Soluble: Methanol, ethanol, dimethyl formamide. Slightly soluble: Benzene, isopropyl alcohol. Insoluble :Water	270.24	$C_{14}H_{10}N_2O_4$	262	75.24	0.67
SB_3	4-(4-hydroxybenzylidene amino)benzoic acid	Light yellow amorphous powder	Soluble: Methanol, ethanol, dimethyl formamide. Slightly soluble: Benzene, isopropyl alcohol. Insoluble :Water	241.24	$C_{14}H_{11}NO_3$	215	73.72	0.45

SS_1	4-{3-chloro-2-[4-(dimethylamino)phenyl]-4-oxoazetidin-1-yl}benzoic acid	Reddish brown amorphous powder	Soluble: Methanol, ethanol, dimethyl formamide. Slightly soluble: Benzene, isopropyl alcohol, chloroform. Insoluble :Water	344.79	$C_{18}H_{17}O_3N_2Cl$	182	65.37	0.71
SS_2	4-[3-chloro-2-(4-nitrophenyl)-4-oxoazetidin-1-yl]benzoic acid	Yellowish amorphous powder.	Soluble: Methanol, ethanol, dimethyl formamide. Slightly soluble: Benzene, isopropyl alcohol, chloroform. Insoluble :Water	346.72	$C_{16}H_{11}O_5N_2Cl$	227	72.34	0.51
SS_3	4-[3-chloro-2-(4-hydroxyphenyl)-4-oxoazetidin-1 yl]benzoic acid	Light yellow amorphous powder	Soluble: Methanol, ethanol, dimethyl formamide. Slightly soluble: Benzene, isopropyl alcohol, chloroform. Insoluble :Water	317.72	$C_{16}H_{12}O_4NCl$	197	70.11	0.66
SS_4	4-{2-[4-(dimethylamino)phenyl]-4-oxo-1,3-	Reddish brown amorphous	Soluble: Methanol, ethanol, dimethyl formamide.	342.41	$C_{18}H_{18}N_2O_3S$	172	69.60	0.47

	thiazolidin-3-yl}benzoic acid	powder	Slightly soluble: Benzene, isopropyl alcohol, chloroform Insoluble :Water					
SS_5	4-[2-(4-nitrophenyl)-4-oxo-1,3-thiazolidin-3-yl]benzoic acid	Yellowish amorphous powder.	Soluble: Methanol, ethanol, dimethyl formamide. Slightly soluble: Benzene, isopropyl alcohol, chloroform. Insoluble :Water	344.34	$C_{16}H_{12}N_2O_5S$	298	75.98	0.62
SS_6	4-[2-(4-hydroxyphenyl)-4-oxo-1,3-thiazolidin-3-yl]benzoic acid	Light yellow amorphous powder	Soluble: Methanol, ethanol, dimethyl formamide. Slightly soluble: Benzene, isopropyl alcohol, chloroform. Insoluble :Water	314.34	$C_{16}H_{13}O_4NS$	248	63.21	0.53

6.4. Screening of antimicrobial activity

The synthesized compounds SS_1-SS_6, were evaluated for their antimicrobial activity by disc diffusion method. As per the data obtained, it was confirmed that all the tested compounds possess antibacterial and antifungal activity against the tested bacterial and fungal strains.

- The synthesized compound SS_1 showed the significant activity in the following order: *Staphylococcus aureus* > *Escherichia coli* > *Candida albicans.*
- The synthesized compound SS_2 showed the significant activity in the following order: *Staphylococcus aureus* > *Candida albicans*>*Escherichia coli.*
- The synthesized compound SS_3 showed the significant activity in the following order: *Escherichia coli* > *Candida albicans* >*Staphylococcus aureus.*
- The synthesized compound SS_4 showed the significant activity in the following order: *Candida albicans*> *Escherichia coli*> *Staphylococcus aureus.*
- The synthesized compound SS_5 showed the significant activity in the following order: *Candida albicans*> *Escherichia coli*= *Staphylococcus aureus.*
- The synthesized compound SS_6 showed the significant activity in the following order: *Candida albicans*> *Escherichia coli*> *Staphylococcus aureus.*

The above observations showed that 2-azetidinone derivatives are found to be more active in inhibiting the bacterial strains and 4-thiazolidinonones were more active against fungal strains.

However, the antimicrobial activity of the synthesized compounds against the tested organisms was found to be less than that of the standard antibacterial drug Ciprofloxacin and standard antifungal drug Ketoconazole at their tested dose level.

Table 2: *In vitro* antimicrobial activity of synthesized compounds by Disc diffusion method

S.No	Compound	Diameter of zone of inhibition (in mm)								
		Staphylococcus aureus			***Escherichia coli***			***Candida albicans***		
		25 µg	100 µg	Std*	25 µg	100 µg	Std*	25 µg	100 µg	Std**
1.	SS_1	18	29.5	32	14	18	29	11	17	30
2.	SS_2	17	28	32	11	13.5	29	14	18	30
3.	SS_3	12	18.6	32	22	27	29	19	22	30
4.	SS_4	10	14	32	11	14.5	29	21	25.4	30
5.	SS_5	11	17	32	12	17	29	23	27	30
6.	SS_6	14	19	32	15	21	29	16	24.3	30

Std* - Ciprofloxacin (100 µg /disc)

Std** - Ketaconazole (100 µg /disc)

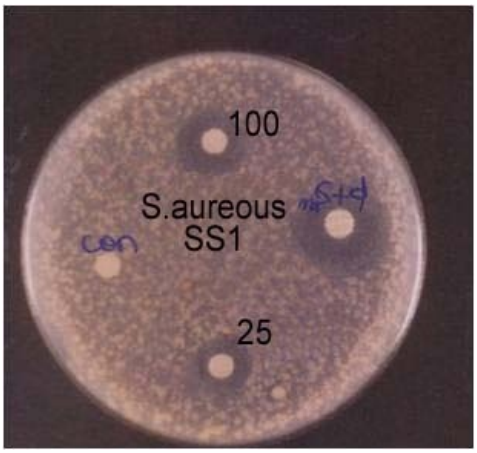


Fig-92: Antibacterial activity of compound SS_1 against *Staphylococcus aureus*

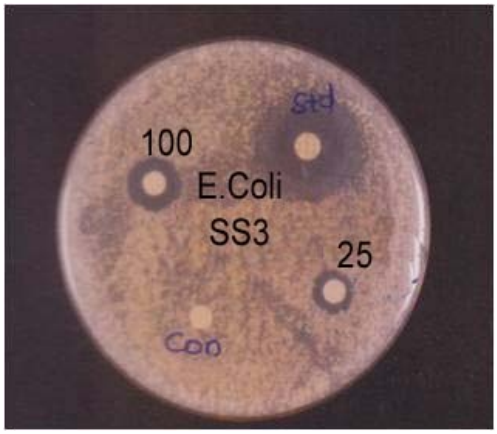


Fig-93: Antibacterial activity of compound SS_3 against *Escherichia coli*

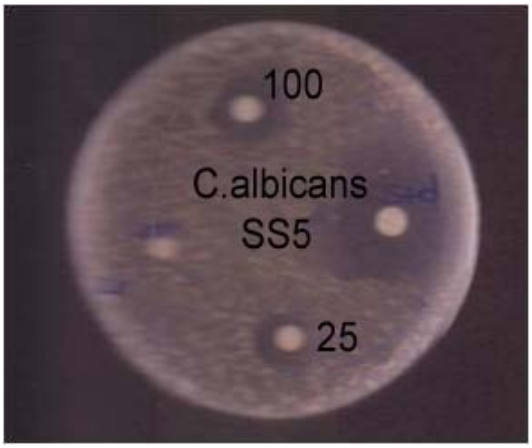


Fig-94: Antifungal activity of compound SS_5 against *Candida albicans*

6.4.a. Acute oral toxicity

The acute toxicity of the synthesized compounds, SS_5 & SS_6 was determined by using swiss albino mice (20-25 g). The animals were fasted for 24 h prior to the experiment and acute class toxic (OECD 423) method of CPCSEA was adapted for acute toxicity studies. The animals were treated up to the dose level of 300 mg and since no mortality was observed up to that level, 1/6th of the lethal dose (50 mg/ kg *b.w*) was taken as effective dose ED_{50}(Therapeutic dose).

6.5. Anti-inflammatory activity

The synthesized derivatives, SS_5 & SS_6 were selected for the screening of anti-inflammatory activity using carrageenan induced paw edema method. The test compounds exhibited significant activity in acute inflammatory models in rats after 1 h, 2 h and 3 h. The results were tabulated in Table-3.

- The SS_5 showed significant activity at 1 h, 2 h and 3 h and the mean paw edema volume at these hours were 0.60 ± 0.057, 0.70 ± 0.057 and 0.81 ± 0.066 respectively. The compound was found to have statistically significant activity at 1^{st} h, 2^{nd} h but it was highly significant in 3^{rd} h.
- The SS_6 showed significant activity at 1 h, 2 h and 3 h and the mean paw edema volume at these hours were 0.56 ± 0.066, 0.66 ± 0.066 and 0.73 ± 0.066 respectively. The compound showed highly significant activity at 3 h, statistically significant at 1 h and 2 h.
- The standard drug diclofenac sodium was significant at 1 h, 2 h and 3 h and the activity at these hours were 0.46 ± 0.066, 0.53 ± 0.088 and 0.53 ± 0.088 respectively, but it was highly significant at 3 h.

Carrageenan-induced hind paw edema was the standard experimental model of acute inflammation. The edema induced by carrageenan injection involves three

phases of chemical mediator release in an orderly sequence (Guang-Qin. *et al.*, **2008**). The first phase (1 h) involves the release of histamine and serotonin and is characterized by increase in vascular permeability. The second phase (2 h) is mediated by release of bradykinin, an important chemical mediator of both pain and inflammation. Release of prostaglandins and cyclooxygenases products takes place in the third and final phase (3 h) (Prakash. *et al*., **2009**).

The compounds produced a significant inhibition of carrageenan induced paw edema at all the three phases. Therefore, it can be inferred that the highly significant inhibitory effect of test compounds on carrageenan induced inflammation could be due to inhibition of the enzyme cyclooxygenase and subsequent inhibition of prostaglandin synthesis. Significant inhibition of paw edema in the early hours of study by compounds could be attributed to the inhibition of histamine and/or serotonin.

The compounds SS_5 & SS_6 exhibited maximum inhibition of 42.31 % and 47.32 % respectively where as the diclofenac sodium showed percentage reduction in edema volume by 62.14 %. From the data it was found that the test compound SS_6 showed superior activity in the inhibition of edema than SS_5. However, both the tested compounds showed less activity than the standard drug.

Table 3: Anti-inflammatory activity by carrageenan induced paw edema model

Group	Dose (mg /kg)	Paw edema volume (in ml) at (mean ± SEM)			
		0 hr	**1 hr**	**2 hr**	**3 hr**
Control	-	0.33 ± 0.066	0.9 ± 0.088	1.1 ± 0.088	1.47 ± 0.057
Diclofenac sodium	20	0.23 ± 0.033	0.46 ± 0.066** (48.88)	0.53 ± 0.088** (51.51)	0.55 ± 0.088*** (62.14)
SS_5	50	0.37 ± 0.057	0.60 ± 0.057* (33.31)	0.70 ± 0.057* (36.32)	0.81 ± 0.066*** (42.31)
SS_6	50	0.31 ± 0.066	0.56 ± 0.066* (37.71)	0.66 ± 0.066** (40.01)	0.73 ± 0.066*** (47.32)

n = 3 in each group.*p< 0.05, **p < 0.01 and ***p<0.001 when compared to control (One-way ANOVA followed by Bonferroni test)

Figures in the parenthesis indicate % inhibition of paw edema

6.6. Analgesic activity

The synthesized derivatives, SS_5 & SS_6 were selected for the screening of analgesic activity using Eddy's hot plate method. The results were shown in Table-4. The compounds showed significant ($p<0.001$, $p<0.01$) analgesic activity at 60, 90 and 180 min.

- The compound SS5, showed significant activity at 90 and 180 min and the latency time was found to be 12.29 ± 0.066 and 11.36 ± 0.577 respectively.
- The compound SS6, showed significant activity at 60, 90 and 180 min and the latency time was found to be 12.13 ± 1.157, 13.14 ± 0.882 and 11.79 ± 0.066 respectively, but the compound showed highly significant activity at 90 min.
- The standard drug pentazocaine was found to have significant increase in latency time 12.87 ± 0.577, 12.91 ± 0.577, 13.65 ± 0.882 and 11.83 ± 1.1 respectively at 30, 60, 90 and 180 min respectively, but the compounds showed highly significant activity at 90 min.

The hot plate test measures the complex response to a non-inflammatory, acute nociceptive input and is one of the models normally used for studying central nociceptive activity. It is an established fact that any agent that causes prolongation of the hot plate latency using this test must be acting centrally. Prolongation of the reaction time in hot plate test signifies that the compounds act through central mechanism. (Zakaria. *et al*., **2008**)

In both test compounds and standard, the percentage analgesia was found to be maximum at 90 min and decreased at 180 min. From these data it was found that the test compound SS_6 (56.80 %) showed superior activity than SS_5 (51.54 %). However, both the test compounds were found to less activity than the standard drug, pentazocine (60.02 %).

Table 4: Analgesic activity by Eddy's hot plate method

Group	**Dose (mg /kg)**	**Reaction time (mean±SEM, sec) at**				
		0 min	30 min	60 min	90 min	180 min
Control	-	7.91 ± 0.577	7.94 ± 0.577	8.01 ± 0.577	8.17 ± 0.577	8.20 ± 0.577
Pentazocine	20	8.53 ± 1.157	12.87 ± 0.577* (50.87)	12.91± 0.577* (51.34)	13.65 ± 0.882** (60.02)	11.83 ± 1.1* (38.68)
SS_5	50	8.11 ± 1.157	11.24 ± 1.157 (38.59)	11.47 ± 1.157 (41.4)	12.29 ± 0.066** (51.54)	11.36 ± 0.577* (40.07)
SS_6	50	8.38 ± 0.066	11.65 ± 1.157 (39.02)	12.13 ± 0.066* (44.74)	13.14 ± 0.882** (56.80)	11.79 ± 0.066* (40.69)

n = 3 in each group *p< 0.01 and **p<0.001 when compared to control (One-way ANOVA followed by Bonferroni test)

Figures in the parenthesis indicate % paw licking/jump response

SUMMARY
&
CONCLUSION

7. SUMMARY AND CONCLUSION

The development of drug resistance has limited the successful application of many antibiotics and therefore this phenomenon has given consequences to health. The design of potential reversers of microbial resistance has thus become a desirable goal in the field of medicinal chemistry. Moreover azetidinone and thiazolidinone are the pharmacophore of various molecules which occupies prominent places in the field of antimicrobial agents, the importance of which are documented in literature.

In the present study, the appropriate aldehydes were reacted with p-amino benzoic acid to give the corresponding a Schiff bases (SB_1-SB_3) which undergoes reaction with chloroacetyl chloride in presence of tri ethylamine results in the formation of corresponding 2-azetidinone derivatives (SS_1-SS_3) by Staudinger reaction. Similarly, the Schiff bases (SB_1-SB_3) reacted with mercaptoacetic acid to give 4-thiazolidinone derivatives (SS_4-SS_6). Both 2-azetidinones and 4-thiazolidinones resulted in good to excellent yield. The purity of the synthesized compounds was checked by appearance of single spot in thin layer chromatography (R_f) and determining melting point.

The structure of the synthesized compounds were established by spectral (IR, ^{1}H NMR and Mass) analysis data. The N=C band (1679-1685 cm^{-1}) and N=CH proton signal (δ 8.52-8.60) in IR and NMR spectrum respectively in the synthesized compounds (SB_1-SB_3), confirmed the formation of Schiff base nucleus. The C=O stretching band (1247-1295 cm^{-1}), C-O-H bending vibration (1408-1437 cm^{-1}) in IR spectrum and a singlet for 1 proton at δ 10.17-10.65 and multiplet for 4 protons at δ 7.15-8.17 in ^{1}H NMR, indicated the presence of benzoic acid group in all the synthesized compounds.

The C=O band (1659-1685 cm^{-1}), CH-Cl band (773-775 cm^{-1}) in IR spectrum and the N-CH proton signal (δ 4.58-4.76) and CH-Cl (δ 5.03-5.50) in ^1H NMR spectrum in the synthesized compounds (SS_1-SS_3), confirmed the formation of 3-chloro-2-azetidinone nucleus. The C=O band (1658-1686 cm^{-1}) and C-S-C band (691-698 cm^{-1}) in IR spectrum and N-CH proton signal (δ 4.52- 4.76) and CH_2-S (δ 7.22-7.36) in ^1H NMR spectrum in the synthesized compounds (SS_4-SS_6) confirmed the formation of 4-thiazolidinone nucleus.

In SB_1, SS_1 & SS_4, showed a singlet for 6 protons at δ 2.91-3.02. and a multiplet for 4 protons at δ 6.60-7.42 in ^1H NMR spectrum and a band at 1434-1456 cm^{-1} for N-$(CH_3)_2$ in IR spectrum, confirmed the substitution of dimethylamino phenyl group.

In SB_2, SS_2 & SS_5, presence of strong bands at 1517 cm^{-1} and 1316-1344 cm^{-1} corresponding to the asymmetric and symmetric O=N=O stretching in IR spectrum and a multiplet for 4 protons at δ 7.07-8.33 in ^1H NMR, indicated the presence of nitrophenyl group.

In SB_3, SS_3 & SS_6, the ^1H NMR showed a singlet for 1 proton at δ 9.57-9.78 and muliplet for 4 protons at δ 6.59-7.42, whereas the IR spectrum of these compounds showed band at 3240-3309 cm^{-1} for OH stretching , indicated the presences of hydroxy phenyl group.

The mass spectrum of the synthesized compounds produced ($M^{+)}$ Molecular ion peaks such as 344.79, 346.72, 317.72, 342.41, 344.34 and 314.34 for SS_1, SS_2, SS_3, SS_4, SS_5 and SS_6 respectively corresponds to their molecular formulas. The predicted chemical structure of titled compounds was further supported by the fragmentation peaks.

Most the synthesized compounds exhibited good activity against the studied set of microorganisms. All the compounds showed good anti bacterial and antifungal activity even at less concentration. The SS_1, **4-[3-chloro-2-[4-dimethylaminophenyl]-4-oxoazetidin-1-yl] benzoic acid** was found to be more active against *Staphylococcus aureus* (gram +ve bacteria). The SS_3, **4-[3-chloro-2-(4-hydroxyphenyl)-4-oxoazetidin-1-yl] benzoic acid** was found to be more potent active against *Escherichia coli* (gram –ve bacteria). The SS5, **4-{2-[4-nitrophenyl]-4-oxo-1, 3-thiazolidin-3-yl} benzoic acid** was found to be active against *Candida albicans.* From the data, it is evident that the 2-azetidinones was more active against the bacterial strains and 4-thiazolidinones were more active against the fungal strains.

The synthesized compounds SS_5 & SS_6 were screened for the anti-inflammatory by carrageenan induced paw edema method and the percentage reduction in the paw edema was measured using plethysmograph. The synthesized compounds are found to be highly significant ($p<0.001$) at 3 h. The compounds SS_5 & SS_6 exhibited maximum inhibition of 42.31 % and 47.32 % respectively where as the standard diclofenac sodium showed reduction in edema volume by 62.14 %.

The analgesic activities of both the synthesized compounds were screened by Eddy's hot plate method. The activity was studied at 50 mg/kg b.w. and their effects were measured at time intervals of 30, 60, 90, and 180 minutes. The synthesized compounds showed significant analgesic activity ($p<0.001$) at 90 min. The maximum analgesic activity and the percentage analgesia of SS_5 & SS_6 was found to be 51.54 % and 56.80 % respectively whereas 60.02 % analgesia was exhibited by the standard drug.

However, in both evaluations, the compounds SS_6, **4-[2-(4-hydroxyphenyl)-4-oxo-1, 3-thiazolidin-3-yl] benzoic acid** showed better activity than SS_5. This may be due to the presence of electron donating group such as hydroxy group at the 2nd position of 4-thiazolidinone than the electron withdrawing group NO_2 group at SS_5.

The synthesized compounds have exhibited significant biological activities; their efficacy is not enough to develop them into clinically useful agents. However these compounds need special attention because of their marked activity. Therefore, the further modification of these compounds is quiet desirable and since acute anti-inflammatory screening and central nociceptive activity was performed, further experiments were needed to elucidate their exact mechanism of activity. It can be concluded that these compounds certainly holds great promise towards good active leads in medicinal chemistry.

BIBLIOGRAPHY

8. BIBLIOGRAPHY

1. Aakash Deep., Sandeep Jain., Probodh Chander Sharma., Sanjeev K. and Manav Malhotra. Synthesis, characterization and antimicrobial evaluation of 2, 5-disubstituted-4-thiazolidinone derivatives, *Arabian Journal of Chemistry*, **2011**, 10, 1-5.
2. Aamer Saeed., Naeem Abbas. and Ulrich Florke. Synthesis and antibacterial activity of some novel 2-arylimino-3-aryl-thiazolidin-4-ones, *Journal of Brazilian Chemical Society*, **2007**, 18(3), 89-93.
3. Abdulla R.F. and Kenneth H.F. Monocyclic antibiotics, *Journal of Medicinal Chemistry*, **1975**, 18, 625-627.
4. Ameya Chavan A. and Nandini Pai R. Synthesis and biological activity of N-substituted-3-chloro-2- azetidinones, *Molecules*, **2007**, 12(11), 2467-2477.
5. Balzarini J., Orzeszko B., Maurin J. K. and Orzeszko A. Synthesis and anti-HIV studies of 2-adamantyl-substituted thiazolidin-4-ones, *European Journal of Medicinal Chemistry*, **2007**, 42, 993-1003.
6. Barreca M.L., Chimirri A., Zappala M. and De Luca L. Discovery of 2, 4-diaryl-1, 3-thiazolidin-4-ones as potent anti-HIV agents, *Bioorganic & Medicinal Chemistry Letters*, **2011**, 11, 1793-1796.
7. Bijo Mathew., Githa Elizebeth Mathew., Nirmal Mathew. and Vijayabaskaran M. Synthesis, characterization of some 2-azetidinone derivatives from 2-aminopyridine and evaluation of their antimicrobial activity, *Der Pharma Chemica*, **2010**, 2(6), 238-242.

8. Cardellini M., Claudi F. and Moracci F.M. Studies on β-lactams- I. stereospecific synthesis of 4-Alkoxy-β-lactams from Imidates, *Synthesis-Stuttgart*, **1984**, 12, 1070-1071.
9. Cecilia Saiz., Chiara Pizzo., Eduardo Manta., Peter Wipf. and Graciela Mahler S. Mircrowave-assisted tandem reactions for the synthesis of 2-hydrazolyl-4-thiazolidinone, *Tetrahedron Letters*, **2009**, 50, 901–904.
10. Chavan A.A. and Pai N.R. Synthesis and biological activity of N- substituted-3-chloro-2-azetidinones, *Molecules*, **2007**, 12(11), 2467-2477.
11. Cunico W., Claudia R.B., Maria de Lourdes G., Liliane Capri R., Solanga Wardell M. S. V. and Marcio Soares. One-pot synthesis of 2-isopropyl-3-benzyl-1,3-thiazolidin-4-ones and 2-phenyl-3-isobutyl-1,3-thiazolidin-4-ones from valine, arenealdehydes and mercaptoacetic acid, *Tetrahedron Letters*, **2007**, 48, 6217-6220.
12. Deepak Pareek A. and Manish Chaudhary. Synthesis and biological evaluation of thiazolidinone derivatives incorporating benzothiazole moiety, *Der Pharmacia Sinica*, **2011**, 2(1), 170-181.
13. Desai N.C., Dave D., Shah M.D. and Vyas G.D. Synthesis and antibacterial activity of some novel 4-oxo- 1,3-thiazolidines, 2-oxo-azetidines and 5-oxoimidazolines, *Indian Journal of Chemistry*, **2000**, 39B, 277-282.
14. Desai N.C., Shah M.D. and Bhavsar A.M. Synthesis and QSAR studies of 4-oxo-thiazolidines and 2- oxo- azetidines as potential antibacterial agents, *Indian Journal of Chemistry*, **2008**, 47B, 1135-1144.
15. Dharmrajan sriram., Perumal Yogeeshwari. and Ashok Kumar T.G. Microwave-assisted synthesis and anti YFV activity of 2, 3-diaryl-1-3-thiazolidin-4-one, *Journal of Pharmaceutical Science*, **2005**, 8(3), 426-429.

16. Freddy Havalder. and Sushil J.M. Synthesis of some azetidin-2-ones and thiazolidinones as potential antimicrobial agents, *Indian Journal of Heterocyclic Chemistry*, **2004**, 13, 197-200.
17. Guang Q.Z., Huang X.D., Wang H., Leungb A.K., Chan C.L., Fong D.W.F. and Yub Z.L. Anti-inflammatory and analgesic effects of the ethanol extract of *Rosa multiflora* Thunb Hips, *Journal of Ethnopharmacology*, **2008**, 118, 290-294.
18. Gududuru V., Eunju Hurh., James Dalton T. and Duane Miller D. Synthesis and biological evaluation of azetidinone and thiazolidinone, *Bioorganic & Medicinal Chemistry Letters,* **2004**, 14, 5289-5293.
19. Hussain Sharba H., Rida Al-Bayati H., Nadjet Rezki. and Mohammed Anouad R. Synthesis of thiadiazoles and 1,2,4-triazoles derived from cyclopropane dicarboxylic acid, *Molecules*, **2005**, 52, 1033-1036.
20. Ishwar Bhat K., Sunil Chaithanya K., Satyanarayana P.D. and Balakrishna Kalluraya. The synthesis and antimicrobial study of some azetidinone derivatives with the *para*-anisidine moiety, *Journal of Serbian Chemical Society*, **2007**, 72(5), 437–442.
21. Itishree Vaidya., Suparna Shukla. and Manasi Chogle. Synthesis of novel 4-thiazolidinone derivatives with anti-inflammatory activity, *International Journal of Current Pharmaceutical Research*, **2011**, 3(4), 85-88.
22. Jieping Z., Blanchet J. and Reeve S. Synthesis of 2-imino-4-thiazolidinone from alkyl trichloromethylcarbinol revisited, a three-component process from aldehyde, chloroform and thiourea, *Tetrahedron Letters,* **2004**, 45, 4449–4452.
23. Jitesh Patel B. and Vikas Desai A. Synthesis of some new thiazolidinone derived from 1-p-tolylethanone and their antibacterial and antifungal activities, *International Journal of Industrial Chemistry*, **2011**, 2(1), 1-8.

24. Joyeau R., Molines H., Labia R. and Wakselman M. N-aryl 3-halogenated azetidin-2-ones and benzocarbacephams, inhibitors of β-lactamases, *Journal of Medicinal Chemistry*, **1988**, 31(2), 370-374.
25. Jubie S., Gowramma B., Nitin K.M., Jawahar N., Kalirajan R. and Gomathy S. Synthesis and biological evaluation of some 3-(methoxy phenyl)-2-aryl Thiazolidin-4-one derivative, *Indian Journal of Pharmaceutical Sciences*, **2009**, 1 (1), 32-38.
26. Jubie S., Gowramma B., Nitin K.M., Jawahar N., Kalirajan R., Gomathy S.and Elango K. Synthesis and antimicrobial evaluation of some 2-Azetidinone derivatives, *International Journal of ChemTech Research,* **2009**, 1(2), 153-157.
27. Kavya Ramkumar., Erik Serrao., Srinivas Odde., Nouri Neamati. HIV-1 integrase inhibitor, *Medicial Research Review*, **2010**, 30, 890-954.
28. Lars J.S., Christopher J., Hobbs Knutsen, Christopher Earnshaw G., Andrea Jenny Gilbert and Sarah Mellor L. Synthesis and SAR of novel 2-arylthiazolidinones as selective analgesic N-type calcium channel blockers, *Bioorganic & Medicinal Chemistry Letters***, 2007**, 17, 662–667.
29. Levinine., Seymaour., Narayanan D. and Venkatachala L, *Chemical Abstract*, 1**971**, 74, 87810.
30. Maaroof Zarei and Masoud Mohamadzadeh. 3-Thiolated 2-azetidinones: synthesis and *in vitro* antibacterial and antifungal activities, *Tetrahedron Letters*, **2011**, 67(32), 5832-5840.
31. Maffi G., Narayanan D. and Venkatachala L, *Chemical Abstract*, **1959**, 53, 20553.

32. Manrao M.R., Gill K.K., Sharma J.R. and Kalsi P.S. Synthesis and antifungal activity of 4-thiazolidinone derivaed from ethylvanillin, *Indian Journal of Heterocyclic chemistry*, **1995**, 5, 85-86.
33. Mehta P.D., Sengar N.P.S., Subramaniyam E.V.S. and Satyanarayana D. Synthesis and biological activity studies of some thiazolidinones and azetidinones, *Indian Journal of Pharmaceutical Sciences,* **2006**, 103-106.
34. Mrunmayee Toraskar., Vithal kulkarni and Vilasrao kadam. Azetidinone: a bioactive moiety, *Journal of Pharmacy Research,* **2010**, 3(1), 169-173.
35. Mulay Abhinit., Mangesh Ghodke. and Nikalje Anna Pratima. Exploring potential of 4-Thiazolidinone- A brief review, *International Journal of Pharmacy and Pharmaceutical Sciences,* **2009**, 1(1), 47-64.
36. Natarajan Ramalakshmishmi., Selvaraj Deepa., Antro Jenny., Gopi., Subramani Arunkumar., Ayarivan Puratchikody and Raj Vijaya Bharathi. Synthesis, characterisation and pharmacological evaluation of novel 4-aryl 3-chloro n-pyridine 2-yl 2-azetidinone, *International Journal of ChemTech Research*, **2009**, 1(4), 1000-1004.
37. Ottana R., Maccari R., Barreca M.L. and Bruno G. 5-Arylidene-2-imino-4-thiazolidinones- Design and synthesis of novel anti-inflammatory agents, *Bioorganic & Medicinal Chemistry Letters,* **2005**, 13, 4243–4252.
38. Pandey V.K., Gupta V.D., Udadhyay M., Singh V.K. and Meenal T. Synthesis, characterization and biological activity of 1,3,4-substituted-2-azetidinone, *Indian Journal of Chemistry*, **2005**, 44B, 158-162.
39. Peter W., Beel H.L. and Stanley C. Synthesis and biological evaluation of azetidinone derivatives, *Chemical Abstract*, **1972**, 76, 140500.
40. Prakash B.N., Pandikumar P. and Ignacimuthu S. Anti-inflammatory activity of Albizia lebbeck Benth., an ethnomedicinal plant, in acute and chronic

animal models of inflammation, *Journal of Ethnopharmacology*, **2009**, 125, 356-360.

41. Raga Basawaraj., Amith L. and VijayKumar T. Synthesis and antitubercular activities of azetidinone and thiazolidinone derivatives from 5-chloro-3-methylbenzofuran, *International Journal of ChemTech Research*, **2010**, 2(3), 1764-1770.
42. Rawal R.K., Tripathi R., Katti S.B. and Pannecouque B.R. Design and synthesis of 2-(2,6-dibromophenyl)-3-heteroaryl-1,3-thiazolidin-4-ones as anti-HIV agents, *European Journal of Medicinal Chemistry,* **2008**, 2, 256-260.
43. Seema Mishra., Srivastave S.K. and Srivastave S.D. Synthesis of 5-arylidene-2-aryl-3-(phenothiazino/benzotriazoloacetamidyl)-1,3-thiazolidine-4-ones, *Indian Journal of Chemistry*, **1997**, 36B, 826-880.
44. Shukla H.K., Astik R.R. and Thaker K.A. 2-phenyl-2-methyl-3-aryl-thiazolidin-4-ones a potential antitubercular compounds, *Indian Journal of Chemical Society*, **1981**, 31, 1182-1183.
45. Srivastava S.K., Asati K.C. and Srivastava S.D. Synthesis of 5-arylidene-2-aryl-3-(benzotriazolo acetamidyl)-1,3-thiadiazol-4-ones as Analgesic and Antimicrobial agents, *Indian Journal of Chemistry*, **2006**, 45B, 526-531.
46. Staudinger H. and Enke F. "Die ketene", *Synthesis-Stuttgart*, **1912**, 141-149.
47. Taranalli A.D., Thimmaiah N.V., Srinivas S. and Saravanan E. Anti-inflammatory, analgesic and antiulcer activity of certain thiazolidinones, *Asian Journal of Pharmaceutical and Clinical Research*, **2009**, 2(4), 209-211.
48. Testa E., Luigi and Fontanella. Synthesis of novel series of 2-azetidinones, *Chemical Abstract*, **1963**, 59, 1561-1569.

49. Udupi R.H. and Jesson M. Synthesis of 1-(2'-carboxy-5'-nitrophenyl)-3,4-substituted azetidin-2-ones as anti-inflammatory and antimicrobial agents, *Indian Journal of Heterocyclic Chemistry*, **1996**, 6, 99-102.
50. Umesh Pratap R., Dhanaji Jawale V., Manisha R. and Ramrao Mane A. *Saccharomyces cerevisiae* catalyzed one-pot three component synthesis of 2,3-diaryl-4-thiazolidinones, *Tetrahedron Letters*, **2011**, 54(14), 1689–1691.
51. Viswajanani sattigeri J., Sonia Ajay., Singhal Smita., Ashok Rattan. and Anita Mehta. Synthesis and antimicrobial activity of novel thiazolidinones, *Arkivoc*, **2005**, 2, 46-59.
52. Wadher S.J., Karande N.A., Sonawane S.D. and Yeole P.G. Synthesis and biological evaluation of schiff base and 4-thiazolidinones of amino Salicylic acid and their derivatives as an antimicrobial agent, *International Journal of ChemTech Research*, **2009**, 1(4), 1303-1307.
53. Zakaria Z.A. and Abdul Gani Z.D.F. Antinociceptive, anti-inflammatory, and antipyretic properties of an aqueous extract of Dicranopteris linearis leaves in experimental animal models, *Journal of Natural Medicines*, **2008**, 62, 187-192.

ANNEXURES

9. ANNEXURES

CERTIFICATE

This is certify that the project title. ACUTE ORAL TOXICITY STUDIES IN MICE FOR SYNTHESIZED 4-THIAZOLIDINONE DERIVATIVES ....has been approved by the IAEC.

S.SHOBA

Name of Chairman/member Secretary IAEC:

Dr. P. Balakrishnamurthy

Name of CPCSEA nominee:

Signature with date

22/7/2011

Chairman/Member Secretary of IAEC:

22.7.11

CPCSEA nominee:

(Kindly make sure that minutes of the meeting duly signed by all the participants are maintained by office)

CERTIFICATE

This is to certify that the project title ..EVALUATION OF ANTI-INFLAMMATORY ACTIVITY OF 4-THIAZOLIDINONE DERIVATIVES..... have been approved by the IAEC.

S.SHOBA
Name of Chairman/member Secretary IAEC:

Dr. P. Balakrishnamurthy
Name of CPCSEA nominee:

Signature with date

S. Shoba 22/7/11
Chairman/Member Secretary of IAEC:

22.7.11
CPCSEA nominee:

(Kindly make sure that minutes of the meeting duly signed by all the participants are maintained by office)

CERTIFICATE

This is certify that the project title.... EVALUATION OF ANALGESIC.....
..... ACTIVITY OF 1-THIAZOLIDINONE DERIVATIVES.....
.....has been approved by the IAEC.

S. SHOBA

Name of Chairman/member Secretary IAEC: nominee:

Dr. P. Balakrishnan

Name of CPCSEA nominee

Signature with date

22/7/11

22/7/11

Chairman/Member Secretary of IAEC:

CPCSEA nominee:

(Kindly make sure that minutes of the meeting duly signed by all the participants are maintained by office)